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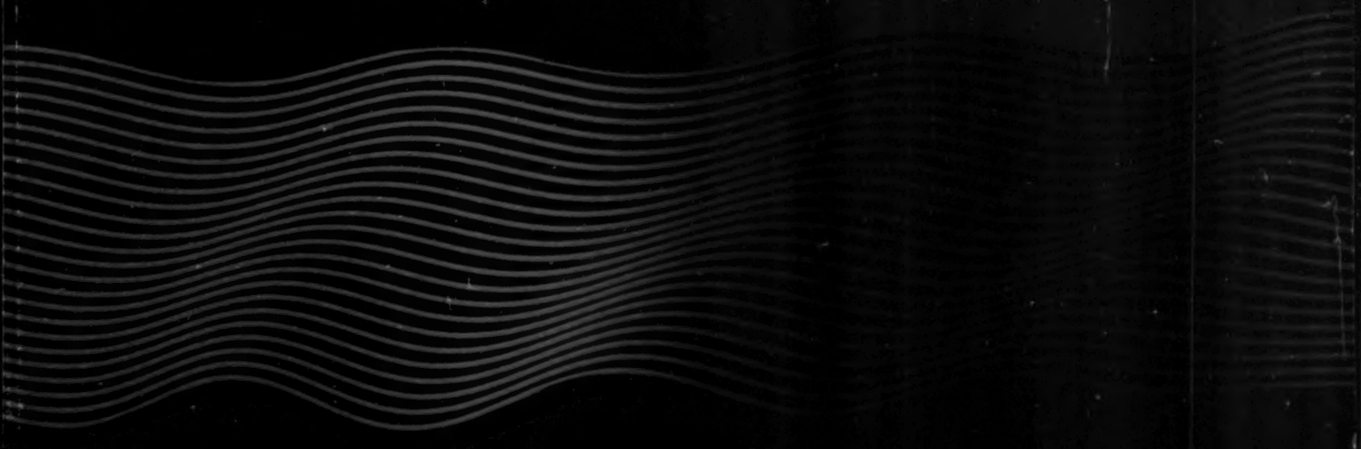

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Acclimation of Rainbow Trout (*Oncorhynchus mykiss*) to Low Environmental pH Does Not Involve an Activation of the Pituitary-Interrenal Axis, but Evokes Adjustments in Branchial Ultrastructure

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Balm, P.H.M., and T.G. Pottinger. 1993. Acclimation of rainbow trout (*Oncorhynchus mykiss*) to low environmental pH does not involve an activation of the pituitary-interrenal axis, but evokes adjustments in branchial ultrastructure. Can. J. Fish. Aquat. Sci. 50: 2532–2541.

Two strains of rainbow trout (*Oncorhynchus mykiss*) were exposed to soft water at pH 4.0 for 14 days, after ambient pH was reduced gradually. Several parameters, either indicators of acid stress, or reportedly involved in the adaptive response to low pH, were monitored. No mortality occurred during the exposure period; feeding behavior, haematocrit, and plasma protein levels were not affected. A transient depression of leucocrit was observed. A minor, but significant, hypochloremia and perturbations in plasma glucose levels occurred in acid-exposed fish from one strain only. There was no evidence of activation of the pituitary-interrenal axis in acid-exposed fish. Baseline plasma ACTH and cortisol levels were indistinguishable from those of control fish, and there was no evidence of sensitization to additional stress in acid-exposed fish. In vitro baseline and ACTH-stimulated cortisol secretion was not significantly different in the two groups. Ultrastructural evidence indicated an increased turnover rate of chloride cells and leucocyte infiltration in gills of acid-exposed fish. These results suggest that interrenal activation and catastrophic ion loss are not inevitable consequences of exposure of rainbow trout to pH 4.0 and that ultrastructural changes in the gills indicate locally regulated adaptive mechanisms.

Deux souches de truite arc-en-ciel (*Oncorhynchus mykiss*) ont été exposées à une eau douce à pH 4,0 pendant 14 jours, après que le pH de l'eau ait été progressivement réduit. Plusieurs paramètres ont été contrôlés, que ce soient des indicateurs du stress d'acidité ou d'indicateurs dont on dit qu'ils participent à la réponse adaptative à bas pH. Il n'y a pas eu de mortalité pendant la période d'exposition; les habitudes alimentaires, l'hématocrite et la concentration de protéines plasmatiques n'ont pas été influencées. Une baisse transitoire du leucocrite a été observée. Une hypochlorémie mineure, mais significative, ainsi que des perturbations de la concentration du glucose plasmatique ont été observées chez des sujets expérimentaux d'une seule souche seulement. Rien n'indiquait une activation de l'axe hypophyso–surrénalien chez les sujets exposés à un bas pH. La concentration de référence de l'ACTH et du cortisol plasmatique ne se distinguait pas de celle des témoins, et rien n'indiquait une sensibilisation à un stress additionnel chez les sujets exposés à bas pH. Il n'y avait pas de différence significative entre les deux groupes au niveau de la sécrétion de cortisol in vitro stimulée par l'ACTH et la sécrétion à son niveau de référence. L'examen ultrastructural a montré un taux de renouvellement accéléré des cellules à chlorures et une infiltration de leucocytes au niveau des branchies de sujets d'expérience. Ces résultats donnent à penser que l'activation interrénale et que la perte catastrophique d'ions ne sont pas des conséquences inévitables d'une exposition de la truite arc-en-ciel à une eau à pH 4,0; en outre, les changements observés à l'échelle ultrastructurale au niveau des branchies pointent vers des mécanismes d'adaptation régulés localement.

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Acidification is a continuing threat to freshwater environments on a global scale. The effects on salmonid populations were first signalled by the results of field studies (reviewed by Leivestad 1982), and subsequently by laboratory studies that confirmed the suggestion that acid challenges are directly related to fish kills. Exposure of rainbow trout (*Oncorhynchus mykiss*), generally considered to be the most acid-sensitive salmonid species (Grande et al. 1978), to low pH waters leads to massive ion losses (in particular sodium and chloride, see reviews by McDonald 1983 and Wood 1989). This effect is ascribed to functional damage occurring in the branchial epithelia (Freda et al. 1991). The resulting ionic imbalances that lead to circulatory failure are believed to be the primary cause of the death of acid-exposed fish (Milligan and Wood 1982; Wood 1989). It has yet to be established to what extent fish can adapt to conditions of low pH. Adaptive ionic regulation in salmonids was first reported by McWilliams (1980), who described the restoration of initially depressed plasma sodium levels in acid-exposed brown trout (*Salmo trutta*, pH 5.0).

Among the regulatory strategies reportedly used by fish under conditions of acid stress is the activation of interrenal cortisol production. Several lines of evidence indicate that in salmonids the interrenal cells are activated in response to environmental acidification. Prolonged elevation of plasma cortisol levels in fish exposed to low environmental pH has been reported in general (see e.g., Brown et al. 1984, 1989). However, in some cases this elevation is only transient (Barton et al. 1985; Goss and Wood 1988) and rarely has been reported not to occur at all (Lee et al. 1983; Witters et al. 1991). These discrepancies may be due to differences in pH, sampling regime, or water chemistry. The suggestion that acidification activates cortisol production is corroborated by histological studies demonstrating increases in interrenal cell nuclear diameter (Brown et al. 1986; Tam et al. 1988), interrenal hyperplasia (Brown et al. 1984; Tam et al. 1988) or increased in vitro incorporation of labelled steroid precursors into cortisol (Sangalang et al. 1990) in acid-exposed trout. In addition, hyperglycaemia, commonly observed during exposure of fish to low pH, has also been interpreted to be an indicator of stimulated interrenal activity under these circumstances (Brown et al. 1989). It has been argued that the fish benefit from the mineralocorticoid actions of cortisol (Balm 1986; McCormick and Bern 1989) when exposed to low pH, especially at the branchial level, where the majority of ions are lost to the water (McDonald 1983; Brown et al. 1989).

It must be emphasized, however, that in all the studies in which plasma cortisol levels were elevated in response to acid exposure, the fish did not adapt in terms of plasma ion concentrations, and consequently the question "do rainbow trout acclimate to low pH?" had to be answered in the negative (Audet and Wood 1988). To our surprise, in a pilot experiment we could not demonstrate disturbance of plasma chloride levels in rainbow trout during a 7-d exposure to low pH (4.0). This prompted us to reinvestigate the effect of low pH per se on rainbow trout in relatively soft lake water. This paper describes the effects of exposure to low pH (4.0, "potentially lethal," Audet and Wood 1988) for periods of up to 2 wk on plasma chemistry and the activity of the pituitary-interrenal axis. Measurements of plasma ACTH and cortisol levels, histological examination of pituitaries and interrenals, and in vitro incubations of interrenals obtained from controls and acid-challenged immature rainbow trout were performed. Because of reports indicating that low pH environments sensitize trout to additional stressors (Barton et al. 1985, 1990), acid exposure was also combined with a confinement stress. Finally, gills of fish exposed to low pH for 14 days were examined by light and electron microscopy, first because the gills are the main route by which ion loss occurs under acid conditions (McDonald 1983), and second because gill tissue is considered an important target for the compensatory mineralocorticoid actions of cortisol under these circumstances (Brown et al. 1989; McDonald 1983).

Methods

Fish

Immature rainbow trout (*Oncorhynchus mykiss*) of two strains (see below) were maintained in 1500-L outdoor tanks, supplied with a continuous flow of lake water at 30 L · min^{-1}. Water composition (micromoles per litre) was: sodium 216, chloride 260, calcium 165, magnesium 46, aluminium 0.2 (total monomeric; measured according to Seip et al. [1984], with slight modifications; detection limit 3 μg · L^{-1}). The water contained minimal amounts of dissolved organic carbon (< 1.0 mg · mL^{-1}). Total alkalinity, 270 μeq · L^{-1}; pH, 7.1; free CO$_2$, 48 μeq · L^{-1}; water temperature, 16 ± 1°C. Fish were transferred to holding tanks 4 wk before the onset of the experiments, and were fed once a day with commercial trout pellets at the manufacturer's recommended rate.

Experimental Design

Two sets of experiments were performed. In the first set (experiment 1, summer 1989) 2+ rainbow trout (Annan strain, mean weight 391 g) of both sexes were maintained at a density of 14 g · L^{-1} (54 fish · tank^{-1}). Tanks were acidified by mixing the water supply to the tanks with dilute H$_2$SO$_4$ at a constant rate by means of a peristaltic pump (Watson Marlow 503U). Mixing took place prior to aeration by a venturi device, so CO$_2$ was driven off before the acidified water entered the tanks, thereby preventing hypercapnic conditions. All surfaces in contact with acidified water were either polythene or glass fibre. The pH was monitored at the outlet of the tanks at regular intervals. Water pH was gradually reduced to pH 4.0 over a period of 6 h, and was maintained at this level for 14 d. At all times during these procedures, great care was taken to avoid disturbing the fish. Seven fish were sampled at 9 a.m. from 2 control and 2 acidified tanks (14 fish per treatment) at 2 and 14 d after the onset of acidification. The experiment was repeated once, and because the data from each of the experiments were statistically indistinguishable, values were pooled to provide a combined sample size of 28 fish at each sampling time. After anaesthetizing the fish in 2-phenoxyethanol (1:2000, Sigma), blood was collected in EDTA/aprotinin (1.5 mg · 3000 KIU^{-1} · mL blood^{-1}, Sigma). Plasma was divided into aliquots for various assays and stored at −20°C. Head kidneys, containing the cortisol producing interrenal cells, were dissected from the fish sampled after 14 d and immediately placed in an incubation medium (Balm 1986). Pituitaries and gill tissue were fixed for light and electron microscopy. The second set of experiments (experiment 2, summer 1991) was conducted with 1+ rainbow trout (Tasmanian strain; all females; mean weight 158 g), kept at a density of 5 g · L^{-1} (48 fish · tank^{-1}). Tank water was acidified in the same manner as in the 1989 experiments. Fish were sampled (8 per tank: 16 per treatment) at 4 h, 2 d, 7 d, and 14 d after the onset of acidification. Sufficient tanks (16) were used in the experimental design to avoid sampling any one tank more than once. In addition, on day 7 and day 14, immediately after removal of the sample group a further eight fish from each tank were subjected to a confinement stress by transfer to a keepnet suspended in each tank. After 90 min of confinement these fish were removed and treated as described above.

In vitro Superfusion of Head Kidneys

Head kidney tissue taken from fish randomly selected from control tanks ($n = 16$) and from tanks acidified for 14 d ($n = 16$; experiment 1) was superfused with medium in incubation chambers (0.4 mL), at a flow rate of 0.03 mL · min^{-1} and 18°C as described by Balm (1986). Each chamber contained tissue from two fish, pooled according to body weight, yielding eight chambers per experimental group. After an initial period in vitro during which cortisol release had declined to baseline levels (180 min), tissue from both control and acid-exposed trout was pulsed with ACTH (hACTH$_{1-39}$ from Peninsula, 30 min 7.5 nmol · L^{-1}). Samples were stored at −20°C until assay of cortisol by radioimmunoassay (RIA) as described by Pickering

TABLE 1. Effects of environmental acidification (pH 4.0) on rainbow trout (*Oncorhynchus mykiss*). Experiment 1: significant differences between means analyzed by Student's two-tailed *t*-test (unpaired design).

		2 d		14 d		
		Controls (28)	pH 4.0 (28)	Controls (28)	pH 4.0 (28)	
Body weight	g	405 ± 14	374 ± 13	393 ± 11	388 ± 15	
Haematocrit	%	35.5 ± 1.0	34.5 ± 1.1	37.4 ± 1.0	33.7 ± 1.0	
Plasma Cl^-	mM	139 ± 2	136 ± 3	131 ± 2	116 ± 2	$p < 0.01$
Plasma glucose	mM	7.6 ± 0.3	8.8 ± 0.6	9.0 ± 0.4	11.6 ± 0.6	$p < 0.01$
Plasma cortisol	$ng \cdot mL^{-1}$	1.6 ± 0.1	3.3 ± 0.8	2.8 ± 0.6	2.4 ± 0.5	

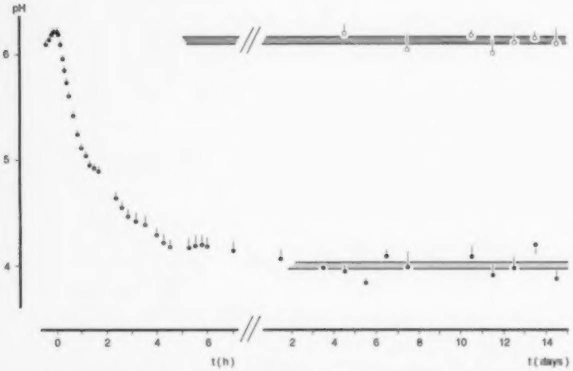


FIG. 1. Experimental water acidification. Data are means ± SE ($n = 4$) from 4 tanks acidified to pH 4.0 (closed circles) and from 4 control tanks (open circles). The shaded areas represent the average pH values ±1 SE for the acidified tanks (4.01 ± 0.04; $n = 4$), and for the control tanks (6.13 ± 0.05; $n = 4$) from day 2 onwards.

et al. (1987). Basal release was defined as the average release rate of three fractions prior to the pulse.

Light and Electron Microscopy

Pituitaries, head kidneys and the first gill arches from control and acid-exposed fish (14-d sample; experiment 1, material from fish selected at random) were fixed in Bouin's fluid, dehydrated in graded ethanol and xylene series, and embedded in paraplast. The total cell volumes of the pituitary ACTH cells and the cortisol-producing interrenal cells was determined as described in Balm (1986). Gill sections (5 μm thickness) were stained with haemalum, eosin, and Alcian Blue. Filament width and lamellar fusion index were determined according to procedures given in Mueller et al. (1991). For electron microscopy, gill filaments (first arch; 14-d sample experiment 1) were fixed as described in Wendelaar Bonga and van der Meij (1989). Thin sections were poststained with lead citrate and examined in a Jeol 100 CX electron microscope. The gills of five fish per experimental group were studied.

Plasma Analysis

Plasma cortisol levels were determined by RIA as described by Pickering et al. (1987). ACTH was determined by RIA according to Sumpter and Donaldson (1986), using the IgG antibody at the recommended dilution. The standard used was obtained from the NIBCS (74/555). The detection limit in our hands was 0.38 pg per tube, and the ED_{50} was 1.9 pg per tube. Glucose and protein levels were measured using commercially available kits (Boehringer Mannheim, and BIORAD 500-002, respectively). Plasma sodium and chloride levels were measured by flame photometric and colorimetric procedures (Technicon). Because Na_2EDTA was used as anticoagulant, the values for plasma sodium were corrected by subtracting a fixed figure (30 mM) from the concentration measured to obtain an estimation of circulating sodium levels. Plasma chloride and sodium levels were compared by regression analysis to detect differences between these ions under the experimental regime.

Statistics

Data are presented as means ±SE ($n = 1$). Overall differences between groups were assessed by subjecting data to analysis of variance (ANOVA, Genstat), with time and treatment (low pH, confinement) as factors. Tanks and fish were used as blocking agents to give a nested-error structure with which to assess the significance of the factors and their interactions.

Results

During the course of both experiments no mortalities were recorded; the low pH exposure had no apparent effect on feeding behavior. Figure 1 shows the rate and magnitude of pH changes observed. These were similar in both experiments, although only the data for experiment 1 are presented. Water pH was gradually reduced to pH 4.0 over a period of 6 h, and was maintained at this value for 14 d. The average pH value recorded in the control tanks was 6.13 ± 0.05 ($n = 4$), which is slightly more acidic than the inlet water. Acidifying the water had no significant effect on the total monomeric aluminium level: 240 ± 30 nM ($n = 4$) versus 330 ± 40 nM ($n = 4$) for the acid tanks (n.s.d.) at 14 d. Table 1 shows the values measured for experiment 1. After 2 d we did not detect differences between the groups, whereas after 14 d significant differences ($p < 0.01$) could be measured in plasma chloride and glucose levels. Analysis of variance, taking all data into account, revealed no effect of pH on body weight, haematocrit, and plasma cortisol, but indicated a significant effect ($p < 0.01$) of low pH exposure on plasma chloride levels. In this experiment, effects on plasma chloride levels were paralleled by changes in plasma sodium levels ($r = 0.86$; $p < 0.000$). None of the parameters studied could be correlated with sex or body weight. After 14 d, the low pH treatment had no influence on the total volumes of either the pituitary corticotropes, or the cortisol-producing cells in the interrenal. Values for total corticotrope volume ($mm^3 \cdot 10^{-4}$) were: 232 ± 20 ($n = 7$) and 226 ± 25 ($n = 8$) for controls and low pH exposed fish, respectively. Total interrenal

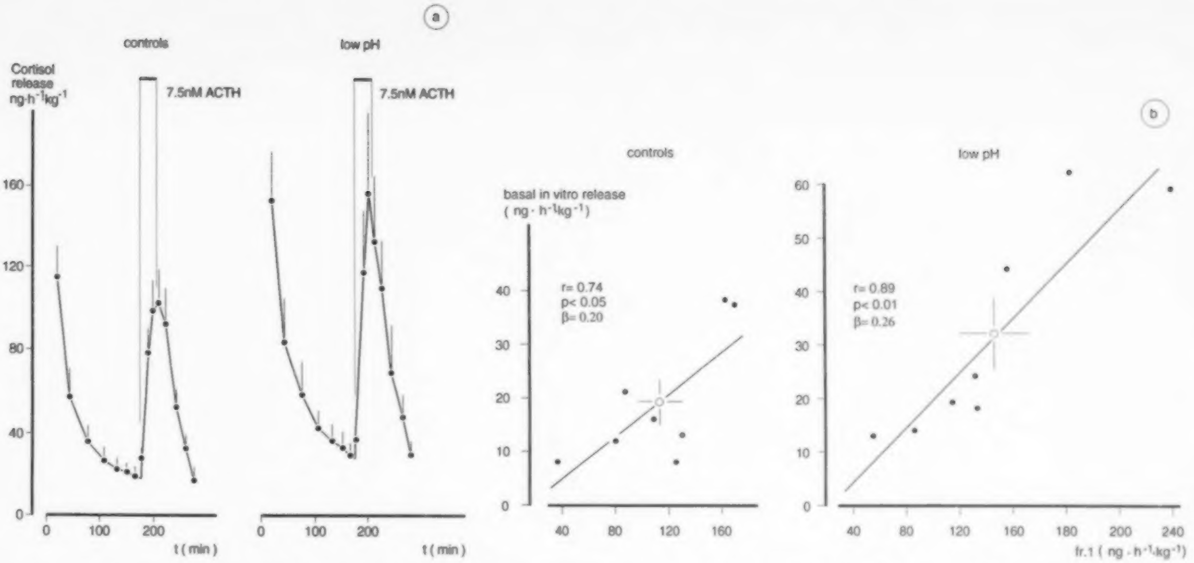


FIG. 2. a) In vitro cortisol release by headkidneys from rainbow trout (*Oncorhynchus mykiss*) exposed to pH 4.0 for 14 d, compared to controls. Data are from 8 individual incubations per group. Cortisol release is calculated as ng cortisol · h^{-1} · kg^{-1} body weight; b) Correlation between initial in vitro cortisol release (first fraction, "fr.1") and in vitro basal cortisol release for control and acid-exposed rainbow trout. Lines were fitted using the method for least-squares linear regression, and the significance of the correlation coefficient (r) was assessed.

TABLE 2. Effects of reducing ambient pH to 4.0 on a number of parameters in rainbow trout (*Oncorhynchus mykiss*), at intervals after the onset of pH reduction (** $p < 0.02$; *** $p < 0.01$). Experiment 2: K factor = coefficient of condition ($100W \cdot L^{-3}$); n.d. not determined; significant differences between means (controls/pH 4.0) analyzed by Student's two-tailed t-test (unpaired design). n = 16 for all experimental groups.

		Body weight (g)	K factor	Haematocrit (%)	Leucocrit %	Plasma protein ($g \cdot dL^{-1}$)
4 h	Controls	157 ± 9	1.16 ± 0.02	37.1 ± 0.8	n.d.	3.3 ± 0.2
	pH 4.0	167 ± 7	1.16 ± 0.02	36.4 ± 1.5	n.d.	3.0 ± 0.2
2 d	Controls	136 ± 9	1.12 ± 0.02	41.1 ± 1.5	2.7 ± 0.3	4.0 ± 0.2
	pH 4.0	158 ± 10	1.13 ± 0.01	40.4 ± 1.7	1.8 ± 0.1***	4.0 ± 0.2
7 d	Controls	173 ± 11	1.18 ± 0.02	38.4 ± 1.2	2.4 ± 0.2	4.1 ± 0.2
	pH 4.0	166 ± 18	1.20 ± 0.02	40.5 ± 1.2	1.7 ± 0.2**	4.3 ± 0.2
14 d	Controls	186 ± 13	1.28 ± 0.06	41.4 ± 1.2	1.8 ± 0.1	3.9 ± 0.2
	pH 4.0	196 ± 9	1.28 ± 0.02	39.0 ± 1.1	1.6 ± 0.1	4.1 ± 0.2

cell volume ($mm^3 \cdot 10^{-4}$) for the controls was 1265 ± 110 (n = 3), versus 1347 ± 80 (n = 3) for the acid-exposed fish. A comparison of the in vitro cortisol release from controls and fish kept at pH 4.0 for 14 d (Fig. 2a) reveals no significant differences. Basal release rates were 19 ± 4 ng · h^{-1} · kg body weight^{-1} (n = 8) and 32 ± 7 ng · h^{-1} · kg body weight^{-1} (n = 8), respectively, whereas the areas under the curves produced by the ACTH stimulation were 287 ± 57 and 383 ± 90, respectively (arbitrary units, n = 8). For both groups there was an —identical— correlation between the initial and basal in vitro cortisol production rates (Fig. 2b).

Experiment 2 (1991), using a different strain of rainbow trout, essentially reproduced the initial findings. In this experiment, there was a time effect on several parameters in the control fish: leucocrit ($p < 0.05$), glucose ($p < 0.01$). Again, no effects of low pH on body weight or haematocrit values were obtained any sampling times (Table 2). The same holds for the coefficient of condition ($100W \cdot L^{-3}$) and plasma protein levels (Table 2). There was an overall effect of low pH treatment on leucocrit values ($p < 0.05$), acid treated fish having a significantly lower leucocrit than controls at 2 d ($p < 0.01$), and 7 d ($p < 0.02$). Figure 3A indicates that baseline plasma levels of cortisol were similar in control and acid-exposed trout throughout the experiment. In addition, there were no significant differences in post-confinement cortisol levels between the two groups at 7 and 14 d (Fig. 3A). Baseline plasma ACTH levels in control and acid-exposed fish were statistically indistinguishable at all sampling times during the experiment (Fig. 3B). Confining acid-exposed and control fish at 7 d resulted in identical elevations in plasma ACTH levels in both groups. At 14 d however, post-stress plasma ACTH levels were lower in the acid-treated trout ($p < 0.02$) than in the controls (Fig. 3B).

The effect of low pH on basal plasma glucose values varied with the length of exposure: acid-treated trout were moderately hyperglycaemic at 2 d ($p < 0.01$), but hypoglycaemic ($p < 0.02$) after 14 d (Fig. 3C). The stress associated with confinement

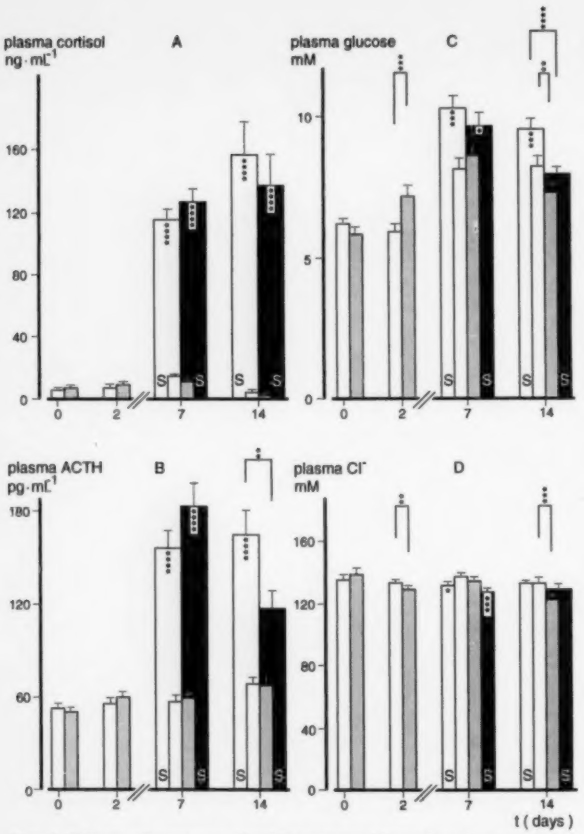


FIG. 3. Effects of exposure of rainbow trout (*Oncorhynchus mykiss*) to pH 4.0 for various periods on plasma cortisol (A), plasma ACTH (B), plasma glucose (C), and plasma chloride levels (D). The narrow bars represent the unstressed groups (open bars controls, $n = 16$, and shaded bars pH 4.0, $n = 16$); whereas the wider bars represent the groups stressed by confinement ("s"; open bars controls, $n = 16$, shaded bars pH 4.0, $n = 16$). The effects of stress are indicated by the symbols in the bars, and differences between corresponding control and pH 4.0 groups are indicated by symbols above the bars. Data from experiment 2. * indicates $0.02 < p < 0.05$; ** $0.01 < p < 0.02$; *** $0.001 < p < 0.01$; **** $p < 0.001$.

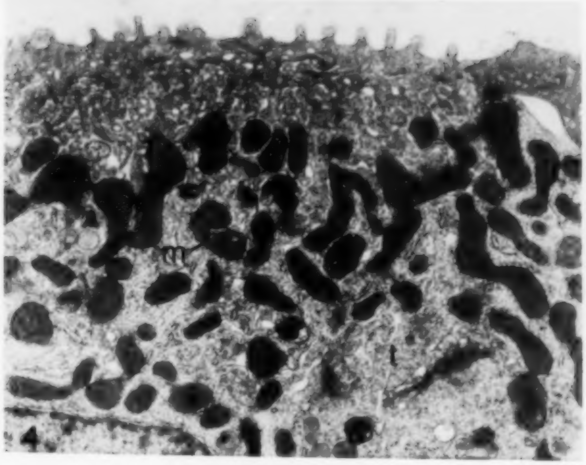
affected the glucose levels of the controls and the acid-challenged trout differently; this was especially evident at 14 d. Confined controls were hyperglycaemic ($p < 0.01$) following stress in comparison to unstressed controls, whereas confined fish exposed to low pH had plasma glucose levels similar to those in their unstressed counterparts. In experiment 2 (1991) a significant overall effect of acid exposure on plasma chloride values could not be demonstrated, in contrast to the results of experiment 1. However, comparing control and low pH trout at 2 d and 14 d yielded small, but significant ($p < 0.02$ and $p < 0.01$, respectively) decreases in plasma chloride associated with the acid-treated fish (Fig. 3D). When the data set was restricted to the 7- and the 14-d samples, stress appeared as a significant factor, reducing plasma chloride concentrations ($p < 0.05$) on day 7 only, in both controls and acid treated fish. In experiment 2, plasma sodium levels were again highly correlated with plasma chloride levels ($p < 0.000$). At 14 d however, the acid exposure had no effect on plasma sodium (168 ± 2 versus 169 ± 2 mM for controls and acid-exposed fish, respectively), despite the significant effect on plasma chloride levels.

At the light microscope level no effects of low pH could be observed (not shown). Filament widths (mm) were 19.8 ± 1.2 versus 19.7 ± 1.0 for controls ($n = 6$) and low pH fish ($n = 6$), respectively. Values for the lamellar fusion index were 2.09 ± 0.04 for controls, and 2.05 ± 0.02 for low pH treated fish. At the electron microscope level, however, several changes were noticeable. The chloride cells, restricted to the filament epithelium in all fish examined, showed an increased structural heterogeneity in the acid-exposed fish. The most frequently encountered chloride cells, under both control and low pH conditions, contained an extensive tubular system, many mitochondria, and an apical membrane in contact with the water over a large area (Fig. 4, 5, 6). These cells were comparable to the mature chloride cells described in tilapia (Wendelaar Bonga et al. 1990). Occasionally small and slender, possibly immature, chloride cells were also found. These were more common in the acid-exposed fish. In the acid-exposed fish, chloride cells often had an electron-dense cytoplasm and a tubular system with an enlarged lumen (Fig. 7, 9). These cells showed the characteristics of degeneration by apoptosis, as described in tilapia (Wendelaar Bonga et al. 1990). In the control fish apoptotic chloride cells were virtually absent. Acid-exposed fish had enlarged intercellular spaces in the epithelium, where leucocytes were frequently found (Fig. 7, 8, 9). Leucocytes were rare in these areas in control fish. Indications of tissue necrosis were as scarce in acid-exposed fish as in the controls, and no clear differences were observed in the ultrastructure of the branchial lamellae.

Discussion

The data presented in this paper demonstrate that, apart from alterations in gill filamental ultrastructure, rainbow trout show very few signs of disturbances resulting from acid exposure per se, and that they can cope with quite severe acidification (pH 4.0) of their environment for at least 2 wk. Minor changes in plasma chemistry were observed, whereas the coefficient of condition, the feeding response, and haematocrit values were not affected. Furthermore, the treatment did not elicit an activation of the pituitary-interrenal axis. These results are surprising in view of the reported negative effects of water acidification on fish generally (Leivestad 1982; McDonald 1983; Wood 1989), and especially because rainbow trout are the most acid-sensitive salmonid species (Grande et al. 1978). There are several reasons

FIG. 4–9. Micrographs of gill filaments from control and acid-exposed trout (*Oncorhynchus mykiss*). Figures 4 and 6 are from controls; Fig. 5, 7, 8, and 9 are from acid-exposed fish. Fig. 4. Mature chloride cell; m, mitochondria; t, tubular system; control (12 000×). Fig. 5. Mature chloride cell; t, tubular system; pH 4.0 (12 000×). Fig. 6. Mature chloride cells (c) and pavement cells (p); no intercellular spaces are present; control (9000×). Fig. 7. Chloride cell (c) in the first stage of apoptotic degeneration; the extended intercellular spaces contain lymphocytes (ly) and a macrophage (mp) with a lysosome (l); pH 4.0 (9000×). Fig. 8. Macrophage (mp), with a large lysosome (l; containing a phagocytosed apoptotic chloride cell) in the intercellular space of the filament epithelium; b, blood lacuna; pH 4.0 (9500×). Fig. 9. Macrophage (mp) with a lysosome-like body (l), probably representing a final stage of lysosomal breakdown of an apoptotic body; p, pavement cell; t, tubular system of a chloride cell, pH 4.0 (9500×).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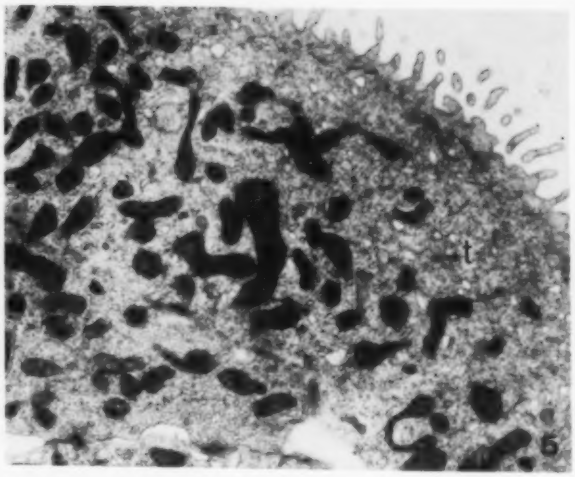
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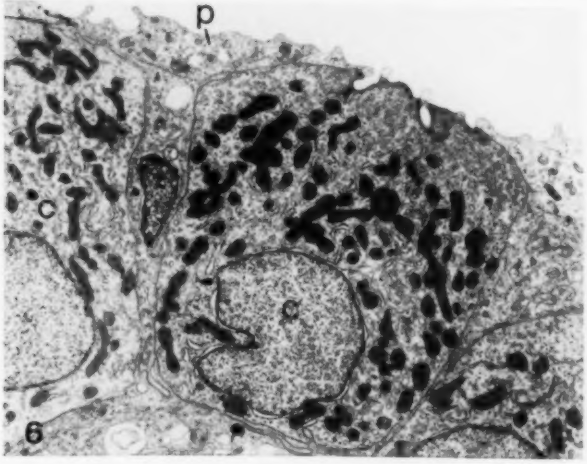
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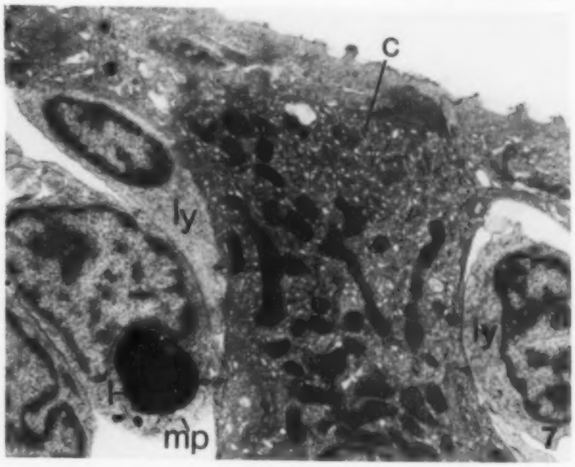
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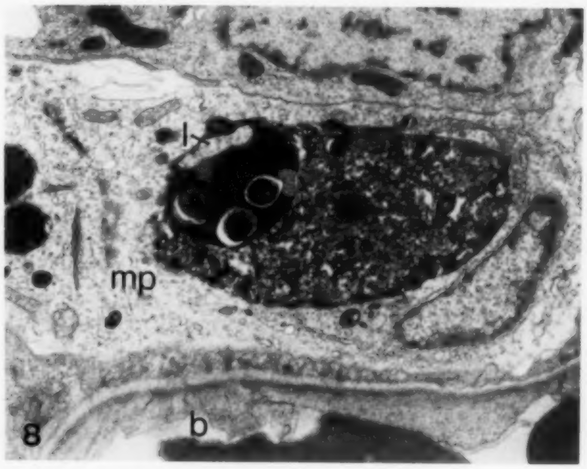
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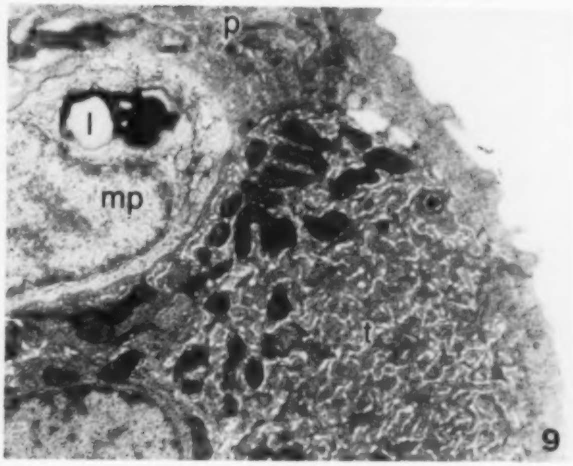
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why we conclude that the experimental conditions have mimicked a "real life" event for the fish, and therefore postulate that the outcome might be generalized for other species.

First, the levels of sodium and calcium in Windermere Lake water indicate that the fish were exposed in relatively soft water. Accordingly, this region has been documented to be susceptible to severe acidification (Turnpenny 1989). In soft water fish face extreme problems in coping with ionoregulatory disturbances, because the external sodium concentration is one of the driving forces in the active uptake of the ion (Maetz 1973), the main compensatory process to counteract losses of this ion to the environment. In addition, calcium ions are essential in maintaining the integrity of branchial tight junctions, which are under threat in the presence of high concentrations of protons (Freda et al. 1991). The fact that at all but one sample point effects on plasma chloride were paralleled by changes in plasma sodium in our fish further substantiates our description of Windermere Lake water as soft, since this coupling is typical of the effects of low pH in softwater environments (Wood 1989). Second, an important characteristic of the water used in our experiments is the virtual absence of aluminium, which allows us to ascribe the effects observed to low pH conditions per se. In many studies on the effects of low pH on fish, the presence of aluminium in the water is unaccounted for (Leino et al. 1987; Wendelaar et al. 1990). The effects of the metal during acidification are complex, and depending on the ambient pH and the parameters investigated, it can further aggravate (Witters et al. 1991), or even ameliorate the effects of low pH (Evans et al. 1988). Third, we used two totally unrelated strains of rainbow trout. The essentially identical results exclude the possibility that we may have used an acid-resistant strain initially. We have no evidence that either strain is acid-resistant, and it is highly unlikely that inherited resistance would underly our results obtained from two geographically distinct strains. Finally, the experiments were carried out during the summer months. Although data demonstrating that there is no effect of the time of year on the susceptibility of salmonids to low pH have been published (Giles et al. 1984), one paper reporting such an effect in fact claims that trout are most sensitive to environmental acidification in the summer (Stuart and Morris 1985). It therefore seems unlikely that the remarkable adaptation we observed can be attributed to a season-related effect.

There are however two major differences in our approach compared to most other studies which we think are crucial, the first being that the water pH was lowered gradually, rather than acutely. The field data available support this approach as environmentally realistic (Henriksen et al. 1984). The importance of this type of regime as a major determinant of adaptational success in laboratory studies has been recognized (Stuart and Morris 1985; Balm et al. 1987; Wendelaar Bonga et al. 1990). Second, the exposure took place in holding tanks where fish were neither transferred nor handled 4 wk prior to the experiment. This is reflected in the low baseline plasma cortisol levels in the fish, typical of other studies carried out at this laboratory (Pickering and Pottinger 1989). Although several studies cited meet one of these criteria, this is the first study on the effects of low pH on fish in which these two methods have been combined.

An overview of the existing literature yields a "low pH syndrome" in fish characterized by increases in haematocrit values and plasma cortisol, glucose and protein concentrations, with concurrent massive ion losses reflected in decreased plasma sodium and chloride concentrations. Indications for these phenomena are commonly reported to occur within 24 h of exposure of trout to pH below 5.0. Of the "typical" responses mentioned we observed only moderate and inconsistent hyperglycaemia and reductions of chloride and/or sodium plasma levels in acid-challenged trout.

The most striking difference between the present results and those reported in the literature is the fact that the apparent acclimation of our fish to the adverse conditions occurred in the absence of an activation of the pituitary-interrenal axis. This has been demonstrated in several ways. Plasma titres of ACTH and cortisol in resting fish did not change in response to acidification. In the literature, plasma cortisol levels in rainbow trout have almost consistently been demonstrated to rise in response to acidification (Ashcom 1979; Brown et al. 1984; Barton et al. 1985; Goss and Wood 1988; Brown et al. 1989), although in some cases this elevation is transient rather than permanent. Importantly, the baseline levels of cortisol in our fish were considerably lower than in most other studies on the effects of low pH. Our data also constitute the first report of plasma ACTH levels in fish exposed to environmental acidification. Resting plasma levels of ACTH in our fish are among the lowest reported to date in fish (Sumpter and Donaldson 1986; Sumpter et al. 1986). In addition, we could not demonstrate hyperplasia of pituitary corticotropes or cortisol-producing interrenal tissue. This also contrasts with histological data on other indicators of interrenal activity. In response to environmental acidification, larger interrenal cell nuclei (Brown et al. 1986), or increased amounts of interrenal cortisol-producing cells (Brown et al. 1984) have been reported. Mudge et al. (1977), who exposed brook trout to pH 4.0 for 1 h only, observed signs of inhibited interrenal cellular activity. Another parameter reflecting interrenal activity in the present study, in vitro cortisol production, also indicated that exposure to low pH had no effect on interrenal function in our fish. In contrast, a comparable in vitro approach (incorporation of a tritium-labelled steroid precursor into cortisol; Sangalang et al. 1990) resulted in the conclusion that low environmental pH stimulated interrenal cortisol-producing cells. Our opposite conclusion is based on the similar basal and ACTH-stimulated in vitro release rates in control and acid-treated trout. The value of our in vitro approach is corroborated by the correlation between circulating cortisol levels and the initial in vitro release rates in our fish (first fraction, $r = 0.61$, $p < 0.02$, not shown). To further substantiate this, the calculated cortisol production rates in our fish, in conjunction with the baseline plasma cortisol levels, are among the lowest reported for freshwater teleosts (e.g., Leloup-Hatey 1974). We suggest that the lower set-point for interrenal cortisol production in our fish, compared to the fish used in other studies on environmental acidification, was a prerequisite for the outstanding ability of our fish to acclimate. It has been well documented that chronic elevation of plasma cortisol levels interferes with a wide range of physiological processes with largely deleterious consequences for fish (Barton and Iwama 1991).

An important feature of the present results is the fact that low pH pre-treatment does not basically alter the reaction of the fish to a confinement stress. In contrast, it has been claimed that trout at acid pH are more sensitive to additional stressors, as indicated by plasma cortisol (Barton et al. 1985), or plasma ion levels (Brown et al. 1990). We cannot explain the absence of a low pH effect on the stress response on the basis of experimental factors, such as stocking density, which has been reported to influence the cortisol response to stress in trout (Vijayan and Leatherland 1990). Our fish were kept at the same density as in the study by Barton et al. (1985), who observed an acid treatment effect on the stress response. Moreover, the present study was done in the season in which trout are claimed to be most sensitive to stressors (such as acidification; Stuart and Morris 1985). We therefore interpret our data as further evidence for a high level of acclimation to acid conditions. Accordingly, no difference in post-stress plasma chloride levels was observed between the control and acid-exposed fish subjected to keepnet confinement, indicating that ionic homeostatic mechanisms allowed the two groups of fish to cope equally well with the additional ionoregulatory burden associated with confinement (Davis and Parker 1983). Only two minor differences were observed between the two groups in reaction to confinement, both at 14 d. First, the elevation of plasma ACTH was lower in the acid-treated trout than in the controls (74 and 143% over basal levels, respectively). However, the cortisol response in reaction to confinement was not significantly different between the two groups. An explanation for this discrepancy could be that the interrenal cells in acid-exposed trout had become sensitized to ACTH, a mechanism that has been observed in tilapia acclimating to low pH conditions (Balm et al. 1987). Because our present in vitro results seem to rule out this possibility in trout, it might be suggested that either the bioactivity of the circulating ACTH had increased or that acid-treated trout rely on other steroidogenic factors in their stress response to confinement. The second difference detected between the two groups of fish at 14 d under stress conditions was in the hyperglycaemic response induced by confinement. In trout, catecholamines, and not cortisol, appear to be the major hyperglycaemic factors under these conditions (Suarez and Mommsen 1987). Indications that acid environments (in either the presence or absence of aluminium) can potentially influence catecholamine metabolism have been presented (Edwards et al. 1987; Witters et al. 1991). Plasma catecholamine levels and data on production and clearance in acid-exposed trout under stress are still lacking.

The blood chemistry of the trout exposed to acid water indicates that ionic regulation in the fish is not affected markedly at pH 4.0, which is in accordance with our observations of the gills with the light microscope. On the other hand, we were able to demonstrate adaptive regulation at the ultrastructural level. Other studies have reported devastating effects of low environmental pH on gill structure, concurrent with physiological malfunctioning (Leino et al. 1987; Evans et al. 1988; Freda et al. 1991). We suggest that the changes observed in our fish are adaptive, and function to maintain the osmoregulatory performance of the gills, as indicated by the plasma chloride levels, both at rest and under stress. Most notably, in the present study, no structural damage or necrosis was found, in contrast to the findings of others (Jagoe and Haines 1990). The higher frequency of immature and apoptotic cells indicates increased turnover of chloride cells. Apoptosis represents physiologically controlled cell death (Altman 1992), and its occurrence in the regulation of chloride cell turnover in acid-exposed fish has been described earlier for tilapia (Wendelaar Bonga et al. 1990). Furthermore, Tietge et al. (1988), who exposed brook trout (*Salvelinus fontinalis*) to low pH, also observed increasing numbers of cells reminiscent of apoptotic chloride cells ("dense cells"), concurrent with larger amounts of normal chloride cells in the branchial lamellae of acid-treated fish. We suggest that the increased turnover rate of chloride cells in our acid-exposed trout serves to maintain the ion transporting capacity of the epithelium under these adverse conditions.

Another striking effect of acidification was the infiltration of leucocytes into the filament epithelium of the acid-exposed fish. A further indication of the involvement of the immune system in the fish studied here was the transient decrease in leucocrit values. Because this decrease occurred in the absence of elevated cortisol levels, it does not simply reflect cortisol-induced immunosuppression. Accordingly, there was no evidence of bacterial or fungal infections in our fish. It has been reported that immunocytes, especially macrophages, are involved in the destruction of apoptotic chloride cells, and thus have a function in the regulation of chloride cell turnover in fish acclimating to low pH (Wendelaar Bonga et al. 1990). We suggest that macrophages perform a similar function in the rainbow trout gill under acid conditions, and that in addition leucocytes infiltrate the tissue to anticipate an invasion of pathogens.

A picture emerges that rainbow trout adapt to the acid challenge primarily by adjusting the structure of the gill epithelium. Regulation of these phenomena might be largely paracrine in nature, as it is clear from the present study that cortisol is unlikely to play an important role in this process. The interrenal activation observed by others in acid treated fish is usually discussed in relation to the mineralocorticoid actions of cortisol, which are of obvious advantage to fish suffering from increased ionic losses. We have demonstrated that this action of cortisol is not a prerequisite for successful acclimation of trout to environmental acidification. In addition to cortisol, other hormones have been reported to regulate gill function in fish (Mayer-Gostan et al. 1987). Of these, prolactin would seem of interest in view of its role in the acclimation of tilapia to low pH conditions (Wendelaar Bonga et al. 1988). However, Fryer et al. (1988), studying the effects of low pH in brook trout, observed severe signs of prolactin cell atrophy in acid-exposed fish. It remains to be investigated whether prolactin or any other regulatory factor is implicated in the adaptation of rainbow trout to low environmental pH.

In conclusion, data have been presented in which two "characteristic" responses to low pH are dissociated. In particular, the near-perfect regulation of branchial function under these extreme conditions, in the absence of interrenal activation, is striking. This is the first unequivocal demonstration that trout can cope with low pH per se, which implies that the field data on fish kills resulting from environmental acidification must be interpreted as evidence for synergistic actions of protons with other abiotic (aluminium) or biotic (micro-organisms) environmental stressors.

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Swimming Speed and Swimming Metabolism of Atlantic Cod (*Gadus morhua*) in Relation to Available Food: A Laboratory Study

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A laboratory study was performed on how young Atlantic cod (*Gadus morhua*) regulate their swimming speed according to available food. A circular tank 15 m in diameter was divided into six sectors, each containing two cod ranging in length from 29 to 42 cm. Live fish 5 cm long on average were used as prey. For the first 48 d food intake was changed every 1 or 2 wk equally for all six groups. During the last 31 d each group received different amounts of food. The mean swimming speed was highest (0.6 bl/s) when food intake was about half the maximum intake and lowest (0.2 bl/s) when food was unlimited or not available. Swimming metabolism was related to food intake in a similar way, but with a larger difference between the most active (50 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) and the least active group (10 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$). Maximum sustained swimming metabolism was estimated to be about 0.6 times the standard metabolism. The estimated energetic cost of swimming as a percentage of energy obtained from the food decreased with food intake from 24% at maintenance ration to 2% at maximum food intake. On a log–log plot swimming speed decreased linearly with time after encounter of prey.

On a étudié en laboratoire comment de jeunes morues franches (*Gadus morhua*) ajustaient la vitesse de leur nage en fonction des proies disponibles. Un bassin circulaire de 15 m de diamètre a été divisé en six secteurs, chacun contenant deux morues de 29 à 42 cm de longueur. Des poissons vivants de 5 cm de longueur en moyenne servaient de proies. Durant les 48 premières jours, la consommation d'aliments a été variée toutes les semaines ou 2 sem de la même façon dans les six groupes. Au cours des 31 derniers jours de l'expérience, chaque groupe recevait une quantité différente d'aliments. La vitesse moyenne de nage était la plus élevée (0,6 longueur $\cdot$ s^{-1}) lorsque les proies données ne correspondaient qu'à la moitié environ de la consommation maximale, et la plus faible (0,2 longueur $\cdot$ s^{-1}) lorsqu'il n'y avait pas de proies ou lorsque les proies étaient fournies à satiété. Il existe une relation semblable entre la partie du métabolisme employée pour la nage et la consommation d'aliments, sauf qu'il existe un écart plus grand entre le groupe le plus actif (50 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) et le groupe le moins actif (10 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$). La partie du métabolisme qui correspond à la nage soutenue maximale a été évaluée à environ 0,6 fois le métabolisme standard. Le coût énergétique estimé de la nage en pourcentage de l'énergie obtenue par les aliments diminue à mesure que la consommation d'aliments augmente, soit de 24 % au niveau de la ration de maintien à 2 % au niveau maximal de consommation. À l'échelle logarithmique, la vitesse de nage diminue linéairement en fonction du temps après la rencontre de la proie.

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In his critical review of metabolism of fish, Winberg (1956) pointed out the need for research on the metabolism of actively moving and feeding fish: ". . . a study of active metabolism of fish should be regarded right now as a top priority objective in the ecological physiology of fish."

Later the use of the tunnel respirometer made it possible to establish the relationship between oxygen uptake and swimming speed (e.g., Brett 1964, 1965; Tytler 1969; Brett and Glass 1973). This apparatus allowed metabolism to be measured during maximum performance and standard metabolism to be measured more accurately by extrapolation to zero swimming speed.

Some other aspects of the energy budget of fish have been studied in detail since the review by Winberg (1956), such as the heat increment or the energy associated with digesting and processing food (Beamish 1974), energy losses in waste products (Elliott 1976) and growth rate (e.g., Brett et al. 1969; Elliott 1975a, 1975b). However, the metabolism of fish in the wild is still virtually unknown, though it has been generally assumed that the metabolic cost of swimming is a major component of the energy budget of active predatory fish. From theoretical considerations the swimming cost of fish searching for prey in the wild has been estimated to be 1–2 times the standard metabolism (Ivlev 1960; Weihs 1975; Ware 1975) or as high as 2–3 times the standard metabolism (Ware 1978; Kerr 1982).

In their review of energetics of fish, Brett and Groves (1979) considered that the next important step was to develop a means to estimate the metabolism of fish in the wild: "The inquiry now stands at the threshold of breaking the barrier of knowledge on metabolic rates of free-roaming fishes, with all their diversity of behavior and energy saving strategies." They concluded that field metabolism could be estimated from detailed measurements of daily activities (swimming speed) of fish in nature, using the relationship between energy expenditure and speed obtained in laboratory experiments.

However, it has proved to be exceedingly difficult to obtain

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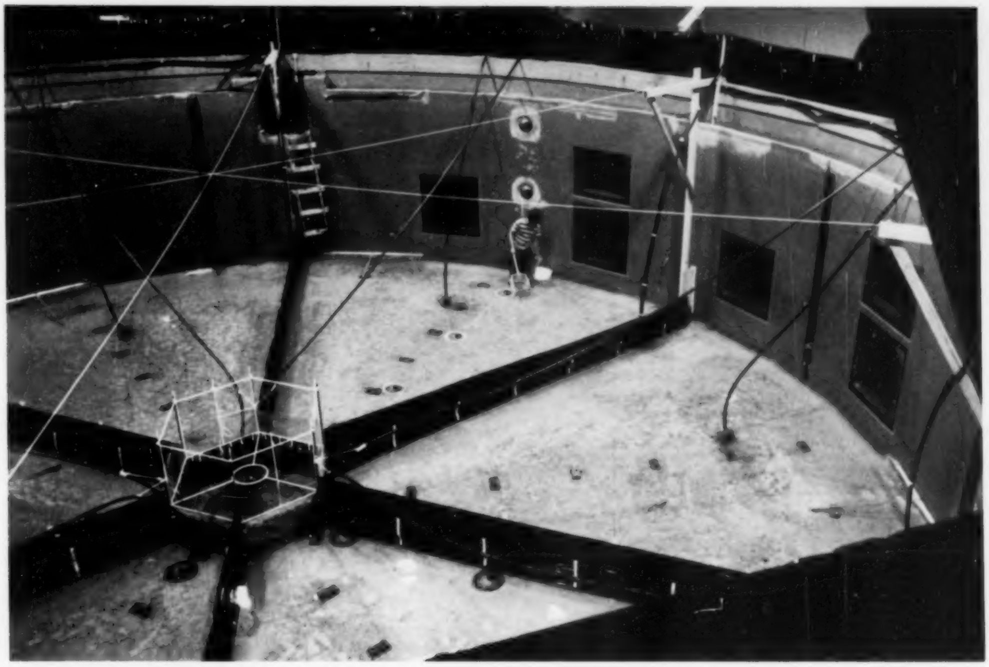

FIG. 1. Experimental tank divided into six feeding areas (30 m^2) each with four prey-dispensing pipes; above plastic sheets to hide observer (photo by Dr. N. Balch).

accurate estimates of swimming speed of fish in nature, particularly in the case of marine fish. There has been some limited success using sonar (Hergenrader and Hasler 1967; Groot and Wiley 1965) or sound transmitting tags (Harden Jones 1977; Stasko and Pincock 1977). The difficulty in estimating the swimming speed of fish in the field indicates that it is useful to study some aspects of this problem in the laboratory. Obviously, the experimental tanks must be much larger than in conventional feeding experiments and live prey must be used. The strength of the laboratory approach is that swimming speed can be measured with great accuracy and food availability can be controlled.

Only in a few laboratory studies has an attempt been made to relate the voluntary swimming speed of fish to the amount of available food. Magnuson (1969), studying tuna feeding on live fish in a large tank, concluded that the effects of foraging requirements on swimming speed could not be separated from the requirements of gill ventilation and hydrodynamic lift. Durbin et al. (1981) found that swimming speed of menhaden increased asymptotically with concentration of phytoplankton.

The main purpose of the present study was to estimate the swimming speed and calculate swimming metabolism of Atlantic cod (*Gadus morhua*) in relation to availability of food. In a laboratory experiment young cod were kept in pairs in large feeding areas. Different levels of daily food intake were maintained for a sufficient length of time to establish a steady state.

Methods

Physical Conditions

A large circular indoor tank, 15.2 m in diameter, at the Aquatron Laboratory, Dalhousie University, Halifax, N.S., Canada, was used in the experiment. The tank was divided into six equal sectors with opaque plastic sheets (Fig. 1). The average depth of the water was 50 cm. A constant flow, 14 L/min, of filtered and chilled sea water entered each compartment. The incoming water was sprayed onto the tank wall and allowed to run down in a thin film to minimize supersaturation. At the center of the tank there was a cage with a drainpipe. The mean temperature was 10.0 ± 0.2°C for the whole experiment and 10.6 ± 0.3°C for the last month of the experiment. Salinity was near 31‰.

Four opaque plastic pipes led into each compartment from the working area above (Fig. 1). The pipes were used to introduce prey into different parts of each compartment, unnoticed by the cod. Several pieces of dark rope were attached around the ends of the feeding pipes to make the entrance of prey less conspicuous. The epiphytes, mainly diatoms, forming irregular brownish patches on the light green bottom of the tank made it more difficult to see the prey. The working area around and above the tank was shielded from the feeding area by opaque plastic sheets to minimize disturbance of the cod, but observations could be made through small peepholes in the sheets.

A fixed 15 h light : 9 h dark cycle was maintained throughout

TABLE 1. Length (L, cm) and weight (W, g) of the cod at the beginning (Aug. 13) and at the end (Sept. 13) of the main experiment. The ration is expressed as a fraction of maximum ration.

Fish	Ration	L_1	L_2	W_1	W_2
1	0	35.2	35.1	344	294
2	0	30.9	30.7	237	209
3	1/8	42.2	42.4	569	555
4	1/8	35.8	36.4	372	358
5	1/4	39.6	39.9	560	564
6	1/4	32.6	33.9	299	342
7	1/2	34.0	35.8	355	416
8	1/2	28.8	31.7	207	276
9	3/4	35.5	37.5	392	512
10	3/4	31.9	35.0	293	438
11	1	36.5	39.8	399	633
12	1	31.1	34.8	248	427

TABLE 2. Daily number and mean weight of prey given to the five pairs of cod during the main experiment (Aug. 13–Sept. 13).

	1/8	1/4	1/2	3/4	1
Daily ration (g)	4.8	10.5	12.7	27.4	42.1
Daily no. of prey	6.9	7.4	8.0	11.2	15.0
Mean wt. of prey	0.7	1.4	1.6	2.5	2.8

the experiment. A gradual change in light level at dawn and dusk (for 1.5 h) was ensured by sequencial switching on and off of the main lamps at certain times combined with an automatic dimming of smaller floodlights. Full illumination at the surface was near 570 lux during the daytime and about 0.5 lux during the dark period, which was just enough light to allow the swimming speed to be measured accurately. To make it easier for the observer to measure swimming speed both during day and night the artificial light cycle was set in such a way that day was between 2 a.m. and 17 p.m. and night between 17 p.m. and 2 a.m.

The Predator and Its Prey

Young cod ranging in mean length from 30 to 42 cm and mean weight from 223 to 562 g were used in the experiment. They were caught near Halifax in June 1980 with small hooks and brought to the laboratory in good condition. Two cod were selected randomly and put in each compartment. They were sufficiently different in length to make individual identification possible (Table 1).

Small, live fish, mummichogs (*Fundulus heteroclitus*), 5 cm long on the average (range: 4–8 cm), were used as prey. A few thousand of these were seined near Cole Harbour, Nova Scotia, and stored in a large tank in the Aquatron Laboratory. The desired ration of prey was prepared for each compartment each morning before their daily feeding with chopped herring. The mummichogs were weighed, counted and stored in separate containers with running sea water and without food until used as prey that same day.

The daily ration of prey was introduced into the tank sometime between 9 a.m. and midnight. Usually the prey were released 4–8 times in each compartment per day at irregular intervals, between one and a few of them at a time. The average number of prey introduced per day varied from 7 to 15 and their mean weight from 0.7 to 2.8 g between compartments during the main experiment (Table 2). To give the prey some time to adjust to the tank they were introduced when the cod was leaving the opening of the selected feeding pipe. The prey were run down the feeding pipe to the feeding area by pouring a small amount of water into the pipe. When restricted ration was given the cod detected and captured the prey within a few minutes. However, at unrestricted ration several prey were available most of the time. They were counted each morning with binoculars to estimate the daily food intake.

The experiment lasted for 79 d (June 27–September 13, 1980). For the first 48 d the daily food intake was changed every 1 or 2 wk for all six groups equally. During the last 31-d period each group received a different amount of food: 0, 1/8, 1/4, 1/2, 3/4, and 1 of maximum food intake, which was about 5% of body weight per day. The length and weight of the fish was measured on days 1, 48, and 79. The caloric content of the prey was measured with a Parr 1341 oxygen bomb calorimeter to calculate the energy intake by the cod (for more details see Björnsson 1985).

Swimming Speed

The method used to measure swimming speed was a modification of that described by Magnuson (1969). Six observation ladders were constructed, one above each sector (Fig. 1–2). A transparent drawing board was attached near the top of each ladder, parallel to the bottom of the tank. By keeping the head in a fixed position against a wooden structure the observer followed the movements of the fish with one eye and traced them on a transparency taped on top of the drawing board. Every 5 s a mark was made on the track at the sound signal of a timer (Fig. 2). No movement for 5 s was indicated with a mark at the side of the preceding mark. Each measurement session lasted for 1 min. Certain types of behavior were noted at the side of the segment in which it occurred. Pens with acetate ink were used to give a permanent record. Four different colors were used to allow four different tracks to be put on the same transparency without confusion. Information concerning the starting position, time, date, sector number, and cod number was also entered on the transparency.

To make sure the observer did not choose a cod of particular activity at any given time a strict measurement routine was always followed: the timer was started as soon as the ladder had been mounted, with each measurement session beginning at the second sound signal; the swimming path was first traced for the larger cod and then immediately for the smaller cod. Furthermore, swimming speed was measured for all six groups in a continuous effort either proceeding clockwise or anticlockwise around the tank. As the encounter of prey clearly affected the swimming speed of the cod, measurements were made as often as possible and at various times after feeding. Normally, there were 4–5 measurement sessions each day, 2–3 during the daytime and 1–2 during the nighttime. During the 79-d experiment over 50 000 swimming speed measurements were made. Most of the measurements were made during the normal working hours (from 8 a.m. to midnight).

The data were digitized as series of coordinates, 3 coordinates for each 5-s segment, using a coordinate reader and digitizer, and the shortest distances between them calculated. Each centimetre on the transparency corresponded to 20.9 cm in the tank at mid-depth. The maximum error would be less than 3% if the fish

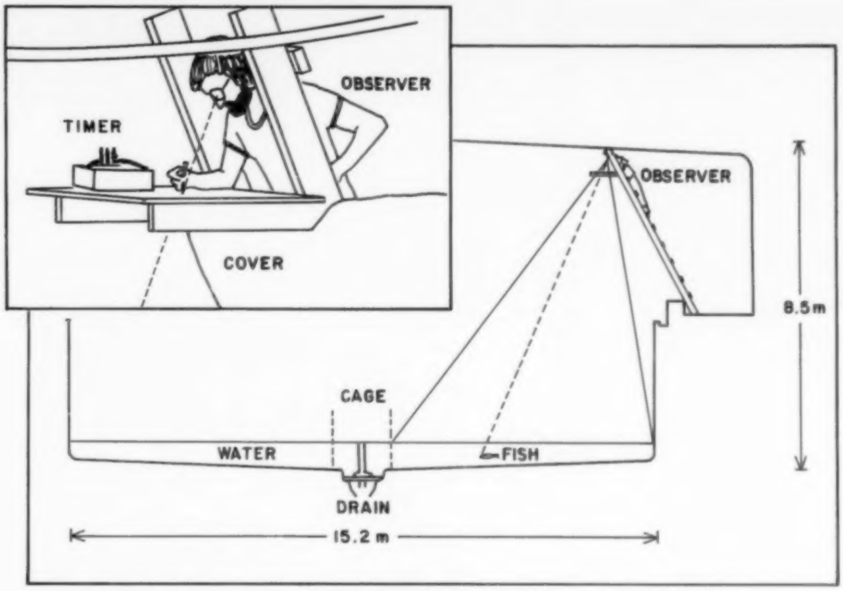

FIG. 2. Diagram of the apparatus and method used to measure swimming speed.

had been swimming at the bottom or at the surface.

To analyze the changes in swimming speed with time throughout the 79-d experiment the data for each cod were grouped into 33 periods, each between 1–4 d long, with 130 measurements in each group on the average (range: 43– 408 measurements).

Swimming Metabolism

All the individual swimming speed (S) measurements were converted to swimming metabolism (T_{sw}) using a published relationship between total oxygen uptake (milligrams O_2 per kilogram per hour) and swimming speed for haddock (Tytler 1969), subtracting the oxygen uptake at zero swimming speed (standard metabolism), as calculated by the same relationship:

$$T_{sw} = 58.9\ 10^{0.33\,S} - 58.9.$$

This relationship, developed for haddock, was selected for lack of data for cod. It should work reasonably well for cod also, because cod and haddock are closely related species and of similar body form. The temperature was similar in both studies and the haddock were only slightly smaller (25 cm) than the cod in the present experiment (35 cm).

Results

Temporal Changes

The experiment consisted of two parts: a preconditioning period, in which all six groups were treated equally; and the main experiment, in which all six groups received a separate food treatment for a long period of time allowing swimming speed to be studied under steady state (Fig. 3). During the period of equal treatment responses to short-term changes in food availability could be studied as well as the individual variation between fish.

In this study swimming speed was expressed as body lengths per second (bl/s) instead of cm/s because a correlation analysis of the data from the preconditioning period indicated that the mean swimming speed of each cod expressed as bl/s was unrelated to its body length ($r = 0.02$, $n = 12$, $P > 0.05$) but increased significantly with body length if expressed in cm/s ($r = 0.73$, $n = 12$, $P < 0.01$).

During the main experiment, activity was highest when food intake was at an intermediate level (Fig. 3). The results of the preconditioning period were in general agreement with this, with two notable exceptions. Firstly, swimming speed was higher during the first than during the second period of unlimited food intake. The cod had been captured only 3 days before the experiment, and thus, it is likely that the fish showed some stressful behavior during the first period, e.g., some of the fish were seen swimming back and forth along the boundaries in the tank. The second exception was the greater swimming speed during the first day of refeeding following 2 wk of starvation. The daily ration of prey was gradually introduced during that day so that the cod were not fully satiated until after the first day. Thus, this transition period in fact corresponded more closely to an intermediate rather than a maximum feeding level.

During the period in which the daily food intake was maintained at 1/4 the maximum intake (days 13–21) the swimming speed increased, becoming 0.56 bl/s on the average during the last 5 d of that period (Fig. 3). There was some lag in the response, probably due to the time required for sufficient hunger to motivate active search. During this period the behavior of the cod became more normal than in the first period, e.g., the swimming back and forth along the sides of the tank became uncommon.

During the 2-wk starvation period the swimming speed decreased in most cases continuously with time, the largest drop in speed being at the start of the experiment (Fig. 3). However, one pair of cod showed increased activity near the middle of the

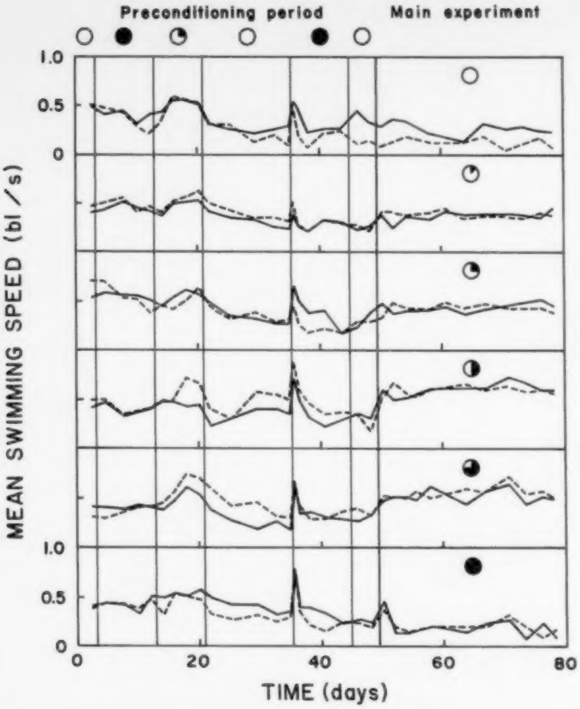

FIG. 3. Changes in mean swimming speed during the whole experiment. Dark area in circles represents the food intake as a fraction of maximum. The larger cod is represented with a solid line, the smaller cod with a dashed line. All scales are equal.

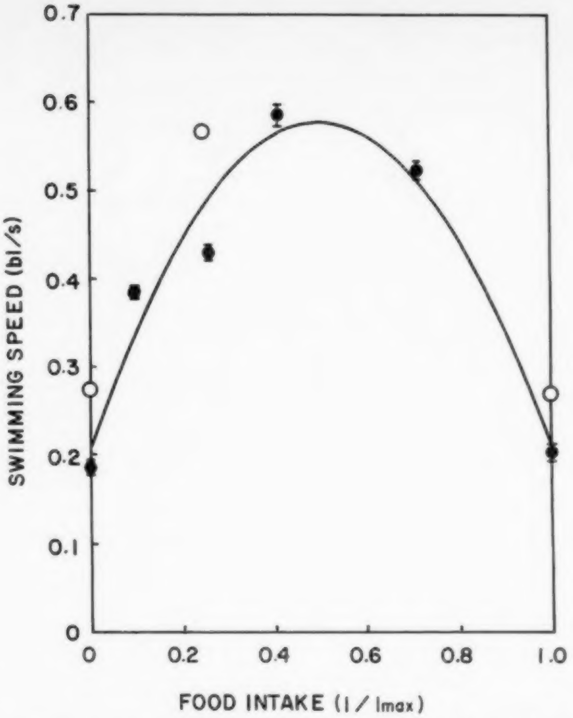

FIG. 4. Swimming speed (S) for each pair of cod during the last 30 d of the experiment in relation to food intake (X). The means with 95% confidence limits are shown (●) along with the parabolic regression ($S = 0.204 + 1.488X - 1.485X^2$, $r^2 = 0.94$, $n = 6$). The mean swimming speed during the last 5 d of each of three ration levels during the preconditioning period is also shown (○). Food intake is presented as a fraction of maximum food intake ($I_{\max}$).

period because one cod developed a popeye and had to be replaced. The substitute cod, of similar size to that removed, had been kept at the same temperature and feeding regime, but in a much smaller tank than in the swimming speed experiment. Apparently, the introduction into a larger tank raised both its own activity and that of the other cod. The mean speed of the other 10 cod was 0.27 bl/s during the last 5 d of this period.

On the first day of refeeding the swimming speed increased sharply, the mean speed varying from 0.4 to 0.8 bl/s between pairs (Fig. 3). As soon as the cod were satiated on the second day of this period the speed dropped to a mean of 0.27 bl/s. After this period feeding was stopped for 3 days before weighing the fish on day 48. The swimming speed remained low during these 3 days.

In the final period of the experiment the mean swimming speed stabilized after 1 d (Fig. 3). The group getting no food and the one receiving unrestricted daily ration had the lowest mean speed (0.2 bl/s). The groups receiving 1/2 and 3/4 of a maximum daily ration had the highest mean speed (0.6 bl/s). The groups receiving 1/8 and 1/4 of a maximum ration swam at an intermediate mean speed (0.4 bl/s).

Sustained Swimming

As a measure of sustained swimming the data for both cod during the last 30 d of the experiment were combined for each of the six groups. The relationship between mean speed and food intake was adequately described by a parabola (Fig. 4). The peak of the parabola was at about half the maximum food intake. The parabolic relationship was further supported by the average swimming speed obtained for three different daily rations during the preconditioning period (Fig. 4).

The frequency distribution of the swimming speed, as measured for 5-s intervals during the last 30 d, showed that the starved fish and the fully fed fish spent a large proportion of their time (35%) not moving (Fig. 5), whereas the fish receiving restricted rations only stopped moving (searching) for a small part of the time (2–5%). The fast swimming speeds, usually associated with encounter of prey, were much more common for the fish receiving restricted ration than for the starving fish or the fish receiving unrestricted ration.

Encounter of Prey

When food was limited, the cod showed some territorial behavior. Usually the larger cod would swim towards the smaller cod and chase it away from its preferred territory, which ranged over more than half of the available bottom area (>15 m^2) including three feeding pipes. Usually the territory of the smaller cod contained two feeding pipes, i.e., one feeding pipe in common (Fig. 1). However, the boundaries were very loose and tended to break up altogether when a prey was encountered, i.e., each of the two cod did not hesitate to go after a moving prey wherever it would go.

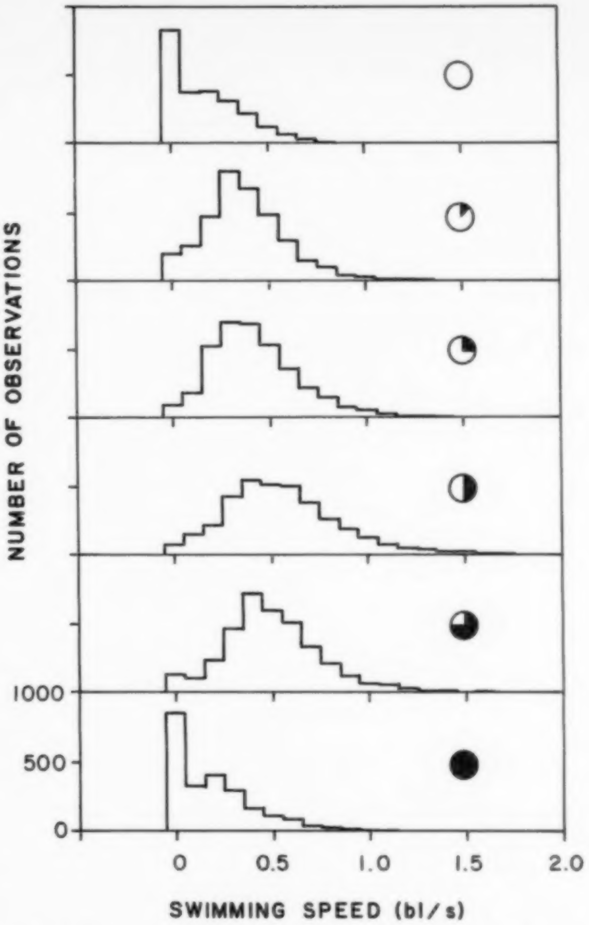

FIG. 5. Frequency distribution of swimming speed during the last 30 d of the experiment for each pair of cod. Dark area in circles represents the food intake. All scales are equal.

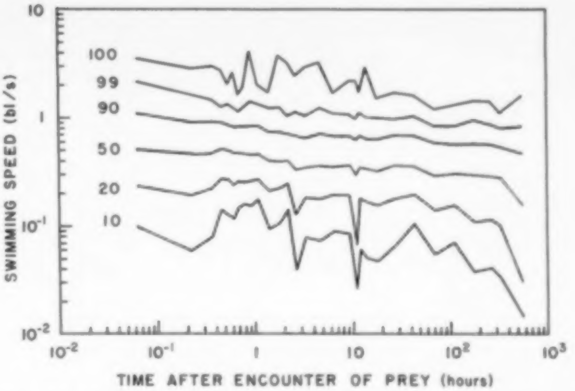

FIG. 6. Selected percentiles of swimming speed related to time after encounter of prey (t_e). All measurements included except those corresponding to unrestricted food intake. The analysis is based on 30 frequency groups of swimming speed corresponding to 30 different intervals of time after feeding.

Soon after a prey had been introduced the cod seemed to detect its smell when entering the area close to the prey. If the prey did not move it was usually not seen by the cod. Normally the cod would then search the area of the smell (<1 m^2) vigorously until it would touch the prey with the pelvic fins or the barbel and then with a quick jerk of the head suck up the prey. On the other hand, if the prey tried to flee, the cod would easily see it, even from a distance of several meters, and immediately dart after it, usually catching it in the first strike by opening the mouth at the right moment thus rushing the water through the gillrakers to capture the prey.

During the experiment it became apparent that the speed of the cod increased abruptly at the encounter of prey and remained high for some time after each encounter. To quantify this, all the swimming speed data, except those corresponding to unrestricted food intake, were sorted with respect to 'time after encounter of prey' (t_e), i.e., the time elapsed from encounter until measurement. These data were subdivided arbitrarily into 30 groups, each containing about 1650 measurements, and their corresponding histograms studied. Then selected percentiles of speed (bl/s) were regressed against t_e. The time of encounter was approximated by the time at which prey was introduced since usually the prey was encountered by a cod within 1 min after entering the tank. A log–log plot of the data gave a near linear relationship (Fig. 6). Correlation analysis showed a highly significant relationship for the selected percentiles ($P < 0.00001$) with a large fraction of the variation in swimming speed being accounted for by a linear regression of the log–log transformed data ($r^2 = 0.49$–0.90). The fast speeds, percentiles 50–99, showed the most regular trend with most of the variation of each being accounted for by a linear regression (79–90%). The slopes of the lines were near −0.1 for all percentiles between 20 and 100 (range: −0.08 to −0.15).

Swimming Metabolism

The difference in mean speed between the most active and the least active group was about 3-fold (Fig. 4). However, the conversion of the swimming speed measurements to swimming metabolism resulted in almost a 4-fold difference in mean swimming metabolism (T_{sw}) between the most active and the least active group (Fig. 7) due to the nonlinearity of the relationship between T_{sw} and speed. Correction for unequal sampling throughout the 24 h period based on regressions between swimming metabolism and t_e (for details see Björnsson 1985) resulted in about 30% higher estimate of maximum swimming metabolism (Fig. 7). As a result, the difference in swimming metabolism between the most active (≈50 mg O$_2$ · kg^{-1} · h^{-1}) and the least active group (≈10 mg O$_2$ · kg^{-1} · h^{-1}) became almost fivefold.

To evaluate the importance of the swimming metabolism relative to other parts of the energy budget it was expressed in multiples of standard metabolism (Fig. 8). The standard metabolism for the experimental cod was estimated from the literature (Saunders 1963; Edwards et al. 1972; Soofiani and Hawkins 1980) to be about 80 mg O$_2$ · kg^{-1} · h^{-1}, applying a correction for temperature and weight (Winberg 1956) to match the conditions in the experiment. Accordingly, the swimming metabolism varied from 0.2 to 0.6 times the standard metabolism depending on the availability of food. The results assuming 25% higher and

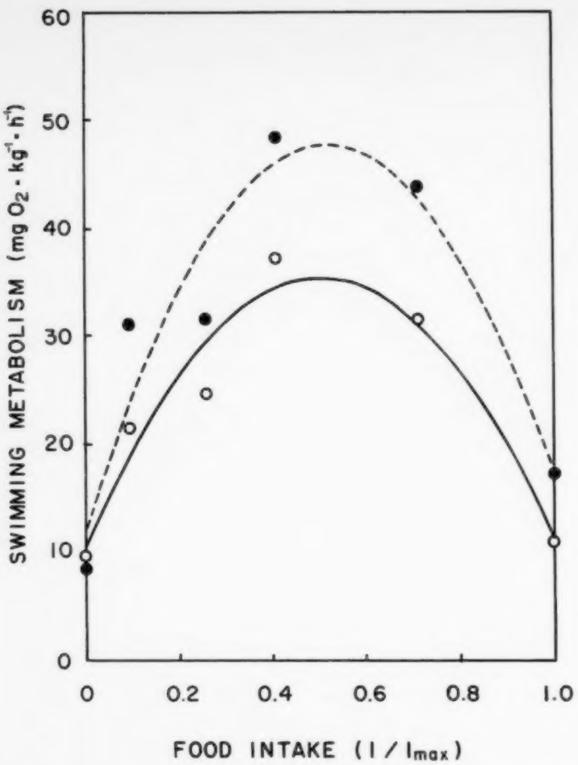

FIG. 7. Swimming metabolism of cod in relation to food intake, without (○) ($T_{sw} = 10.1 + 99.7X - 98.6X^2$, $r^2 = 0.94$, $n = 6$) and with (●) ($T_{sw} = 11.5 + 138.5X - 132.4X^2$, $r^2 = 0.90$, $n = 6$) correction for unequal diurnal sampling effort.

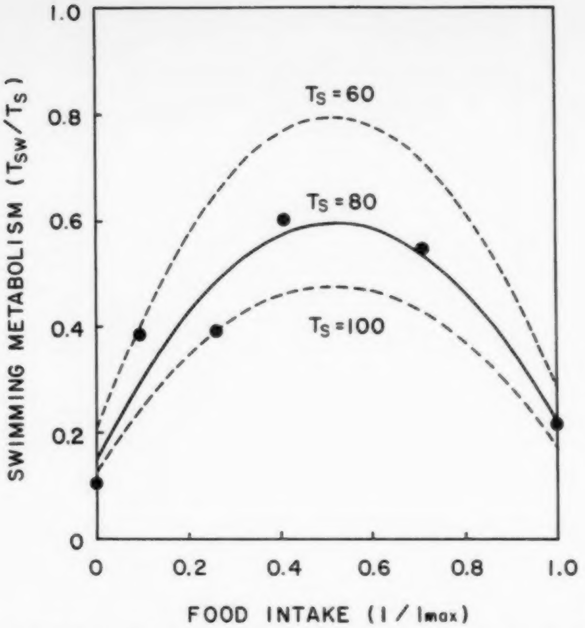

FIG. 8. Swimming metabolism (T_{sw}), corrected for unequal sampling, shown as multiples of standard metabolism (T_s) in relation to food intake for $T_s = 80 \pm 20$ mg $O_2 \cdot kg^{-1} \cdot h^{-1}$.

lower standard metabolism are also shown in Fig. 8.

The swimming metabolism was also expressed as a fraction of total food intake (Fig. 9), assuming that each calorie in the food was equivalent to 0.3085 mg O_2 (Brett and Groves 1979). The functional form in Fig. 9 is a modification of the corrected parabolic relationship (Fig. 8). The swimming metabolism presented in this way declined sharply with food intake from 24% at maintenance ration to 2% at maximum ration (Fig. 9).

Discussion

Sustained Swimming

The dome-shaped relationship between swimming speed and food intake of cod (Fig. 4) suggests that energy saving is of major importance for cod when food is scarce but as the food conditions improve more foraging effort is justified. The results also show that the fast speeds, the ones requiring most energy, were reduced the most with reduced availability of food (Fig. 5), which is in accordance with an energy saving strategy (Ware 1975). However, as the fish became satiated their incentive to forage decreased (Holling 1966; Beukema 1968).

Both the starved cod and the ones getting plenty of food spent a large part of their time motionless at the bottom or at the side of the tank, at which time some of them formed irregular stripes across their backs as a camoflage. By remaining relatively still while digesting the food, the fish may increase their digestion rate, minimize their energy expenditure on swimming, and reduce their risk of predation. However, the continuous swimming of the cod when the availability of prey was limited (Fig. 5) suggests active search by this predatory fish is crucial.

The present experiment is the only one in which swimming speed of a predatory fish has been studied in relation to different availability of prey under steady state conditions. However, in two studies changes in voluntary swimming speed of fish have been related to short-term changes in availability of food (Magnuson 1969; Durbin et al. 1981) and yet others have some bearing on the subject (Ivlev 1960; Hunter and Thomas 1974; Jones 1978; Baily and Yen 1983). Results from these studies seem to be consistent with the general dome-shaped relationship described here for cod.

Magnuson (1969) found that the swimming speed of tuna (*Euthynnus affinis*) in a large tank declined with time after feeding, the decrease being most rapid at first. His interpretation was that swimming activity appeared to be more closely associated with requirements to maintain hydrostatic equilibrium and gill ventilation than for food search. However, the similar trend observed for cod can not be explained in this way, because cod do not have to swim continuously to avoid sinking or to obtain enough oxygen. Therefore, a more likely explanation, at least for cod, is an energy saving strategy when food is scarce. During part of the study by Magnuson tuna were kept in the tank with thousands of small fish as prey. Swimming speed was only slightly higher during the day than during the night. He concluded that this observation was not consistent with requirements to regulate swimming activity for food search, since tuna only fed during the day. However, most of the time the cod in the present experiment also showed little reaction to prey when they

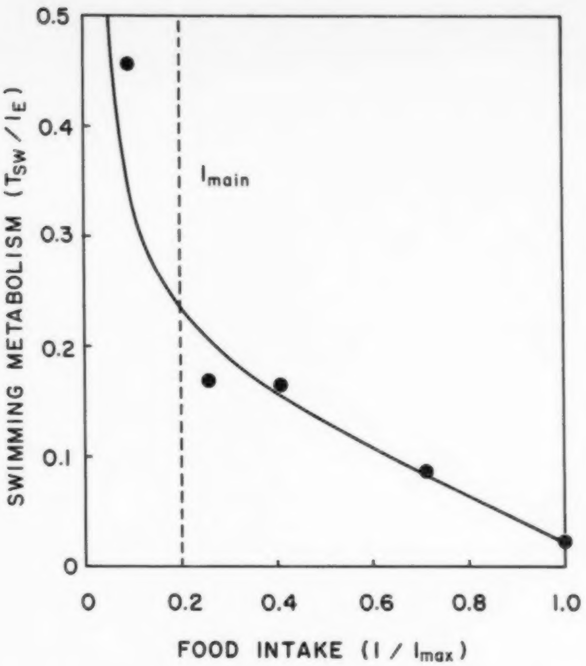

FIG. 9. Swimming metabolism (T_{sw}), corrected for unequal sampling, shown as a fraction of energy intake (I_E): $T_{sw}/I_E = (11.5/X + 138.5 - 132.4X)/I_{max}$, where X is the food intake as a fraction of maximum food intake (I/I_{max}). Maintenance ration (I_{main}) indicated with a dashed line.

were plentiful, which may be explained by reduced hunger and thus lack of incentive to forage (Holling 1966; Beukema 1968). Magnuson also found that the response of tuna to food flavors increased with the time they were deprived of food suggesting that tuna increased swimming speed when food concentration was high enough to stimulate active foraging but low enough to sustain hunger, as observed for cod in the present experiment.

Durbin et al. (1981) found that the swimming speed of menhaden (*Brevoorita tyrannus*), a filter-feeding fish, increased asymptotically with concentration of plankton in the water. However, each experiment only lasted 24 h with plankton made available only for 7 h. The menaden were starved for 36 h prior to each experiment. Therefore, even the menhaden receiving the highest concentration of plankton were never fully satiated with food.

According to Jones (1978), the average swimming speed of cod and haddock in a laboratory experiment ranged from 0.21 bl/s at 6°C to 0.34 bl/s at 13°C. The level of food intake was not noted and the experimental conditions were not described in detail, but his results are similar to the lowest speeds observed in the present experiment for starved and satiated cod. Jones noted that when the fish were released into an area where food had been planted at low densities a swimming speed of 1.3 bl/s was observed at first, but during feeding it dropped to about 0.6 bl/s. These results are consistent with the dome-shaped relationship between swimming speed and daily food intake observed for cod in the present experiment.

The activity of larval fish has been reported to decrease with increased concentration of food (Ivlev 1960; Hunter and Thomas 1974) and to decrease with the degree of starvation (Baily and Yen 1983). Similarly, the medusa *Aurelia aurita* L. doubled its swimming speed after the capture of one fish larva, but decreased its swimming speed after the capture of many fish larvae (Bailey and Batty 1983). All these results are consistent with the dome-shaped relationship observed for cod (Fig. 4).

It is concluded that the dome-shaped relationship between mean swimming speed and daily food intake observed for cod in the present experiment may be a generality applying for a variety of unrelated species which actively hunt for their food.

Encounter of Prey

The finding that swimming speed of cod increases at the encounter of prey is consistent with studies of several other aquatic animals (Magnuson 1969; MacKay 1976; Durbin et al. 1981; Bailey and Batty 1983), suggesting that there is an important advantage to such a response. Undoubtedly, foraging behavior may comprise some of the most important evolutionary traits of fish and other animals, since natural selection favors a predator which uses an optimal foraging strategy (Ware 1975, 1978; Krebs 1978).

Regulation of foraging speed by a predatory fish may be of major importance since swimming is costly, and an optimal forager should allocate energy when and where it is most profitable. If the distribution of food were even or random it seems that an optimal predator should move at a constant speed except perhaps for the brief moments between encounter and capture. However, it is clear that the activity of the cod in the present experiment remained high long after capture of encountered prey, suggesting an adaptation to clumped distribution of prey. It is well known that food in the ocean is very patchy and laboratory experiments have shown that fish can forage more efficiently when the distribution is clumped that when it is even (Ivlev 1961; Lasker 1975).

According to the present experiment swimming speed decreased linearly (on a log–log plot) with t_e. It is intriguing that such a simple relationship seemed to apply for a time scale from 4 min to 25 d. The slopes were similar for the different percentiles, which means that the relative decrease in speed over time (t_e) was similar for both high and low speeds. However, the absolute decrease in speed increased with increased percentiles, i.e., the fastest and the most costly speeds were used mainly when chances of obtaining food were greatest, which is consistent with an optimal foraging strategy. Fry (1957) noted that the resting metabolism of fish declines linearly with the length of a starvation period on a log–log scale. Possibly, part of this decrease was due to reduction in swimming metabolism, as observed in the present study.

Swimming Metabolism

A few workers have from theoretical considerations estimated the foraging metabolism of an active predatory fish. The estimate by Weihs (1975) was 0.9 times the standard metabolism, estimates by Ivlev (1960) and Ware (1975) were as high as 1–2 times, and those by Ware (1978) and Kerr (1982) as high as 2–3 times the standard metabolism. The estimate of swimming metabolism of cod in the present experiment, 0.2–0.6 times the standard metabolism, was considerably lower.

The swimming speed measurements for cod in the present experiment are quite accurate; the maximum measurement error was less than 5%, and the estimated sampling bias due to unequal sampling was maximally 30%. Furthermore, there was some uncertainty involved in calculating swimming metabolism from swimming speed measurements. The calculation was based on Tytler's (1969) data for 25 cm haddock weighing 156 g. The cod in the present experiment were on the average 35 cm long, weighing 388 g, i.e., 2.5 times heavier than the haddock. However, the results by Brett (1964, 1965) indicate that weight-specific swimming cost in fish is not very sensitive to an increase in body weight. An increase in weight of sockeye salmon from 50 to 1432 g (a 29-fold increase) only caused a 35% increase in the weight-specific cost of swimming (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) at 1 bl/s and even less at lower speeds.

There also appears to be little difference in the cost of swimming between haddock and sockeye salmon for a given size and temperature (Tytler 1969; Brett 1964, 1965). The difference in swimming cost should be even less between cod and haddock which are more similar morphologically, ecologically, and genetically than haddock and sockeye salmon. Therefore, it seems unlikely that a substantial bias was caused by using the haddock relationship for cod.

In the experiment each measurement of swimming speed was made for a time interval of 5 s and therefore the fastest burst speeds associated with prey capture by the cod would be underestimated by this method. However, these fast speeds were only associated with a part of all the prey captures, i.e., when the prey tried to flee from the cod. More often than not the prey did not move and relied on not being seen. In those cases fast speeds were not required. The average number of prey caught by each cod was only about 4 per day and thus explosive feeding attacks occurred only rarely. Instead, most of the swimming by cod was of the socalled cruising speed type. Thus, it seems unlikely that the estimate of daily swimming metabolism would have increased substantially with a greater resolution than the 5 s used in the experiment.

It is generally believed that a fish of similar size and shape and at similar temperature as the cod in the present experiment cruising at 1 bl/s expend about an equal amount of energy on swimming as on standard metabolism (Brett 1964, 1965; Tytler 1969; Weihs 1975; Ware 1978). In no given period during the 79-d long experiment did the mean swimming speed of any cod exceed 1 bl/s (Fig. 3), and in the case of the most active group during the last month of the experiment only about 10% of the measurements were above 1 bl/s (Fig. 5). Therefore, it seems clear that the swimming metabolism of cod was relatively low in the present experiment.

However, there is some question about how well these results apply to a cod in the wild. Although each feeding area in the experiment (30 m^2) is large for a laboratory experiment it is certainly small compared to the space available for a cod in the sea even though tagging experiments indicate that juvenile cod are very stationary and do not normally undertake any long distance migrations until later in life (Svåsand and Kristiansen 1990). Furthermore, the foraging speed may to some extent depend on the species of prey and its size and behavior. Still, it seems likely that the dome-shaped relationship here described will apply, at least qualitatively, to cod in nature and the estimate of swimming metabolism may at least serve as a first approximation for the free-roaming cod.

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Changes in Behaviour of Atlantic Halibut (*Hippoglossus hippoglossus*) and Turbot (*Scophthalmus maximus*) Yolk-Sac Larvae Induced by Bacterial Infections

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Skiftesvik, A.B., and Ø. Bergh. 1993. Changes in behaviour of Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) yolk-sac larvae induced by bacterial infections. Can. J. Fish. Aquat. Sci. 50: 2552–2557.

Eggs of Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) were exposed to *Flexibacter ovolyticus* and pathogenic *Vibrio* sp. strains prior to, and during hatching. Activity, buoyancy and mortality of the yolk sac larvae were monitored from hatching until time of first feeding. The halibut larvae showed reduced activity and increased mortality in response to the challenge of bacteria, compared to uninfected control groups. In addition, the infected halibut larvae showed increased specific density compared to the uninfected larvae. These responses were not found for turbot. However, turbot larvae infected with *Vibrio anguillarum* had lower activity than larvae infected with *F. ovolyticus.* The reduced activity of halibut larvae occurred 1–2 weeks prior to the increased mortality, allowing infections to be detected at an early stage. The results suggest that the behaviour of fish larvae is influenced by bacterial infection.

Les oeufs de flétan (*Hippoglossus hippoglossus*) et de turbot (*Scophthalmus maximus*) ont été exposés à *Flexibacter ovolyticus* et des souches pathogènes de *Vibrio* sp., avant et pendant l'éclosion. L'activité, la flottabilité et la mortalité des larves vésiculées ont été contrôlées de l'éclosion au premier repas. Par comparaison à leurs groupes témoins non infectés, les larves de flétan avaient une activité réduite et une mortalité accrue du fait qu'elles étaient exposées aux bactéries. En outre, les larves de flétan infectées étaient plus denses que les larves non infectées. Ces réponses n'ont pas été observées chez les larves du turbot. Toutefois, parmi ces dernières, les larves qui étaient infectées par *Vibrio anguillarum* étaient moins actives que les larves infectées par *F. ovolyticus.* L'activité réduite des larves de flétan a été observée une à 1–2 sem avant que la mortalité ne s'accroisse; il est ainsi possible de détecter les infections très tôt. Les résultats donnent à penser que le comportement des larves de poisson est influencé par les infections bactériennes.

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Regulating buoyancy and activity is the means by which pelagic fish larvae influence their spatial distribution (Pittman et al. 1990b), which eventually influences their potential to survive. Infections by different pathogens are often associated with characteristic changes in host behaviour (Dobson 1988, Dawkins 1990 and references therein). Microorganisms are intimately associated with the mucosal surfaces of the skin and intestine of fish larvae (Yoshimizu et al. 1980; Sugita et al. 1982; Campbell and Buswell 1983; Olafsen 1984; Muroga et al. 1987; Sugita et al. 1988; Hansen et al. 1992 a, b; Olafsen and Hansen 1992). In the case of halibut larvae, some of these microorganisms have been shown to be opportunistic pathogens (Bergh et al. 1992).

If pathogens cause significant changes in the buoyancy and behaviour of marine fish larvae, they may indirectly influence the spatial distribution of the larvae in their natural environment as well as in aquaculture systems. In aquaculture, a pathogen-induced shift in behaviour might provide a suitable way of detecting infections at an early stage, thereby making it possible to discard infected larvae. In the natural environment such a possible mechanism would constitute a hitherto unrecognized indirect pathway by which pathogens influence the potential for survival of their hosts. The purpose of the present study was to investigate whether opportunistic pathogens are capable of changing the behaviour of their hosts.

Materials and Methods

Bacteria

The following bacterial strains were used: *Flexibacter ovolyticus* NCIMB 13127 (National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland), isolated from halibut eggs (Hansen et al. 1992a); *Vibrio* sp. HI-10448, isolated from halibut; and *Vibrio anguillarum* HI-11360, isolated from turbot (O.M. Rødseth, Institute of Marine Research, Bergen, Norway personal communication). *Flexibacter ovolyticus* was used to infect both halibut and turbot; *Vibrio* sp. HI-10448 was used in the experiments with halibut; and *V. anguillarum* HI-11360, with turbot. All bacteria were cultured on Difco 2216 Marine Broth

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(Difco, Detroit, USA) with 1.5% Bacto agar (Difco) added, and suspended in 25 ppt autoclaved seawater immediately before infection.

Halibut Experiments

The halibut eggs were reared in 250-L upwelling incubators, at 7–8°C until three days before hatching. They were then transferred to polystyrene multidishes (Nunc, Roskilde, Denmark). Each multidish consisted of 6 identical wells, 35 mm in diameter and 18 mm high. Each well contained 11 mL autoclaved 25 ppt seawater. One egg was transferred to each well. The infection and further incubation was performed according to Bergh et al. (1992). Two days before hatching the eggs were infected with the various bacteria. Groups infected with *F. ovolyticus* were denoted FA, FB and FC. The groups infected with *Vibrio* sp. HI-10448 were denoted VA, VB, and VC. Each infected group consisted of 60 larvae, each in an individual well. To provide enough larvae to allow for simultaneous video recordings of infected groups and control groups, the control group consisted of 180 larvae, each in an individual well. Three different concentrations of each bacterium were used, approximately $1.0 \cdot 10^6$ (Groups FA and VA), $5.0 \cdot 10^5$ (Groups FB and VB) and $1 \cdot 10^5$ (Groups FC and VC) bacteria $\cdot$ mL^{-1}, as verified by epifluorescence counting. One day after hatching, 10 mL of water was removed from each well and 10 mL autoclaved 25 ppt seawater was immediately added.

The eggs/larvae were incubated in darkness in a temperature-controlled room at 5°C. Mortality was recorded until 34 d after hatching.

The larvae were filmed on days 3, 9, 13, 24, and 33 after hatching. They were filmed under red light with an intensity of 2.5 lux with a Philips L DH 670 video camera. One multidish of the control group, one multidish of the *F. ovolyticus*-infected group and one multidish of the *Vibrio* sp. HI-10448-infected group were filmed simultaneously for 30 min, thus 18 larvae were filmed simultaneously. The camera was mounted approximately 1 m above the multidishes. All wells of the 3 multidishes were included in the viewfield of the camera. After 30 min, the 3 multidishes were replaced by other multidishes, until all had been filmed. Activity was measured from the video recordings. In this study, activity is defined as the percentage of the time the larvae showed any movement that was visible on the video recordings during the last 15 min of the 30-min recording period for each well. In addition, the percentage of larvae that showed any movement during the 15-min observation time was measured.

For buoyancy measurements, two different batches of eggs were used, one infected with *F. ovolyticus* and one infected with *Vibrio* sp. HI-10448. The challenge doses were approximately equal to VA and FA (above), and each group consisted of 60 larvae. In addition, one control group of 60 larvae was used for each batch of eggs.

Turbot Experiment

The turbot eggs were obtained from a commercial hatchery (Øye havbruk, Øye, Norway). The eggs were brought to the laboratory one day before hatching and immediately transferred to polystyrene multidishes (Nunc, Roskilde, Denmark). These multidishes had 24 identical wells, each 15.8 mm in diameter and 18.0 mm high. Each well contained 2 mL autoclaved 25 ppt seawater. One egg was transferred to each well. The eggs were divided into four groups. Each group consisted of 144 larvae. Two groups were infected with *F. ovolyticus*, one (*F. ovolyticus* 1) immediately after transfer to the polystyrene dishes, and one (*F. ovolyticus* 2) immediately after all eggs had hatched. One group was infected with *V. anguillarum* HI-11360, immediately after transfer to polystyrene dishes. A fourth group served as a control. The concentrations of bacteria were approximately $1 \cdot 10^6$ bacteria $\cdot$ mL^{-1} in the wells for each of the three infected groups. Seventy-two of the 144 larvae from each group were checked daily for mortality, and filmed on days 1, 3 and 5. The remaining 72 larvae from each group were used for buoyancy measurements.

The eggs/larvae were incubated and filmed in white light at 117.4 lux. Mortality was recorded until 6 d after hatching. Each multidish was filmed for 20 min on days 1, 3, and 5 after hatching. Four multidishes were filmed simultaneously, one control, and one of each of the three infected groups. After 20 min, the 4 multidishes were replaced by others, until three multidishes from each group (72 larvae) had been filmed. The camera was mounted approximately 1 m above the multidishes, and all wells of the four multidishes were included in the viewfield. Activity was measured as the percentage of time that the larvae showed any visible movement, during the last 10 min of the 20-min video recording period. In addition, the percentage of larvae that showed any movement during the 10-min observation time was measured.

Measurements of Buoyancy

Larval buoyancy was measured for both species by their position in a buoyancy column, according to Coombs (1985). The column consisted of a glass cylinder of 45-mm internal diameter and a height of 70 cm with a salinity gradient and internal standards. For buoyancy measurements, 10 larvae from each group were anaesthetized in their wells by addition of 100 μL of a 1 $g \cdot L^{-1}$ stock solution of methomidate chloride (Hypnodil™ Janssen Pharmaceutica, Belgium) in distilled water. After transfer to the buoyancy column, the specific density of the larvae was calculated from their position relative to the standards, by a computer programme developed by Dr. A. Mangor-Jensen, Austevoll Aquaculture Research Station. No larvae were used repeatedly for this purpose. Buoyancy measurements were carried out on days 7, 9, 14, 17, 21, 23, and 28 (halibut infected with *Vibrio* sp. HI-10448); days 7, 10, 14, 16, 21, and 23 (halibut infected with *F. ovolyticus*); and days 1–6 (turbot experiment).

Results

Mortality

All groups of infected halibut larvae had significantly higher numbers of mortalities during the experimental period than the control group (Fig. 1; $q > 11.62$, $p < 0.001$, Tukey-type multiple comparison of proportions, Zar 1984), with the exception of group VC ($q = 4.327$). A dose-response relationship was found for the groups infected with *Vibrio* sp. HI-10448. No such dose-response was found for the groups of larvae infected with *F. ovolyticus*; the group exposed to the highest concentration of bacteria showed the lowest mortality.

In the experiment with turbot larvae, *F. ovolyticus* did not cause mortality significantly different from that in the control group (Fig. 2). The group infected with *V. anguillarum* HI-11360, however, showed a significantly higher mortality than all other

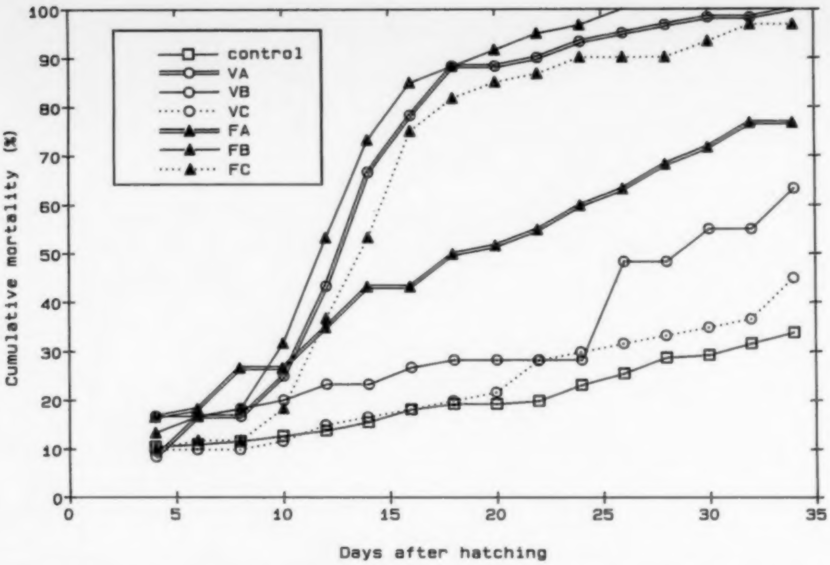

FIG. 1. Cumulative mortality (%) of halibut larvae. Groups infected with *Flexibacter ovolyticus* are denoted FA, FB and FC, whereas the groups infected with *Vibrio* sp. HI-10448 are denoted VA, VB, and VC. Each group consisted of 60 larvae each in individual wells, except the control group that consisted of 180 larvae each in individual wells. Three different concentrations of each bacterium were used, approximately $1.0 \cdot 10^6$ (Groups FA and VA), $5.0 \cdot 10^5$ (Groups FB and VB) and $1.0 \cdot 10^5$ (Groups FC and VC) bacteria · mL^{-1}.

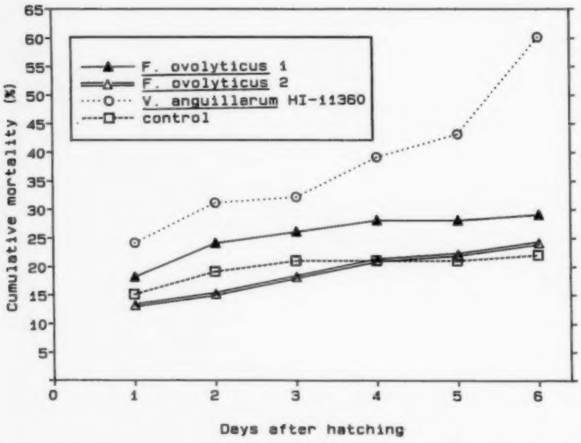

FIG. 2. Cumulative mortality (%) of turbot larvae. Group *F. ovolyticus* 1 was infected immediately after transfer to polystyrene dishes, whereas group *F. ovolyticus* 2 was infected immediately after all eggs had hatched. One group was infected with *V. anguillarum* HI-11360, immediately after transfer to polystyrene dishes. A fourth group served as a control. The concentrations of bacteria were approximately $1 \cdot 10^6$ bacteria · mL^{-1} in the wells for each of the three infected groups. Each group in this part of the experiment consisted of 72 larvae.

groups at the end of the experiment ($q > 6.58$, $p < 0.001$, Tukey-type multiple comparison of proportions, Zar 1984).

Activity

Those halibut larvae in the control group that survived throughout the experiment showed an activity approximately one order of magnitude higher than all groups that were infected with varying concentrations of *Vibrio* sp. HI-10448 or *F. ovolyticus* (Fig. 3a). Activity was significantly lower in all these groups compared to the control group, at day 3 ($p < 0.001$, Robust *t*-test of medians, Hoaglin et al. 1983). In addition, the fraction of infected larvae that showed any measurable activity (Fig. 3b) was significantly lower than that in the uninfected group ($\chi^2 = 59.98$, $p < 0.001$). Of all the larvae that showed any measurable activity at day 3, 30.9% died during the experimental period, compared to 89.0% mortality among those that did not show any measurable activity at day 3. The difference is significant ($\chi^2 = 37.15$, $p < 0.001$).

The *F. ovolyticus*-infected larvae could not be significantly separated from the *Vibrio*-infected larvae with respect to activity. After day 13, however, the activity of the remaining larvae in the *F. ovolyticus*-group was zero. The characteristic decrease in activity at day 8 which was found for both the control group and the *Vibrio*-infected group is typical of halibut larvae kept in darkness (Skiftesvik et al. unpublished data).

No difference in mean activity could be found for turbot (Fig. 4a). However, at day 1, significant differences in the fraction of the active larvae were found ($\chi^2 = 17.33$, $p < 0.001$), as a higher fraction of both groups of *F. ovolyticus*-infected larvae were active (Fig. 4b). At day 5, differences in the fraction of active larvae were found ($\chi^2 = 13.05$, $p < 0.005$) as a significantly lower fraction of the *V. anguillarum* HI-11360-infected group was active than in the *F. ovolyticus*-infected groups

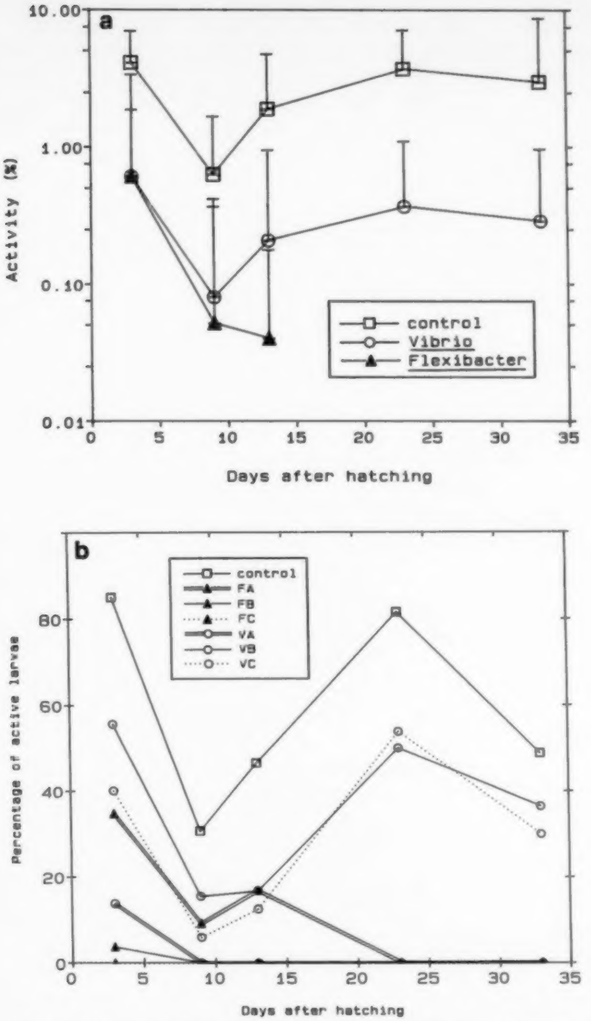

FIG. 3. a) Mean activity of the halibut larvae (percentage of time that movement was detected). In the control group, only those larvae that survived throughout the experiment are included; b) Percentage of active halibut larvae. All larvae that were alive at the time of sampling in each group are included.

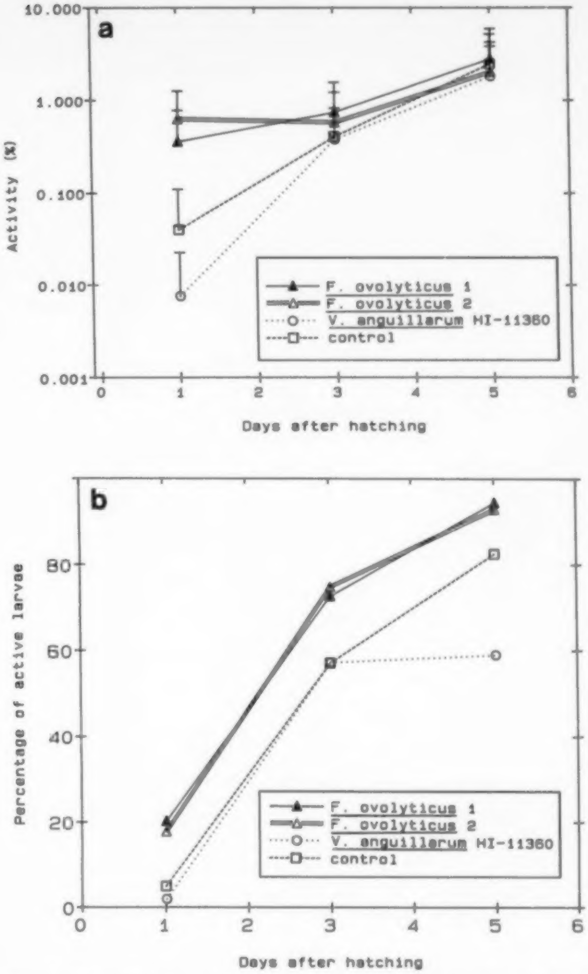

FIG. 4. a) Mean activity of the turbot larvae (percentage of time that movement was detected). All larvae that were alive at the time of sampling in each group are included; b) Percentage of active turbot larvae. All larvae that were alive at the time of sampling in each group are included.

($q \geq 4.6$, $p < 0.01$ Tukey-type multiple comparison of proportions, Zar 1984). However, the control group could not be significantly separated from either of the infected groups.

Density

The specific densities of halibut larvae infected with *Vibrio* sp. HI-10448 and those infected with *F. ovolyticus* (Fig. 5) were not significantly different from their respective control groups at the beginning of the experiment. However, from day 9 to day 17 the *Vibrio* sp. HI-10448-infected group had significantly ($p < 0.048$, Student's *t*-test) higher specific density than its control group (Fig. 5a). A similar significance ($p < 0.011$, Student's *t*-test) was found only at day 14 for the *F. ovolyticus*-infected group (Fig. 5b). No significant differences were found thereafter.

The specific density of the turbot larvae was approximately 1.020 $g \cdot cm^{-3}$ at the beginning of the experiment, slowly increasing to the range 1.021–1.025 at day 6. The group infected with *V. anguillarum* HI-11360 had significantly ($p < 0.0035$, Robust *t*-test of medians, Hoaglin et al. 1983) lower specific density at day 2, but otherwise, the control group could not be significantly separated from any of the infected groups (results not shown).

Discussion

The present results demonstrate a behavioural response to bacterial infections in halibut and turbot larvae that has so far not been recorded in research on fish larvae. Activity is a reflection of metabolic state and influences the energy requirements of larval fish (Laurence 1977). As we have shown that activity was affected by bacterial infections, such infections might influence the results from measurements of activity, and thus the energy

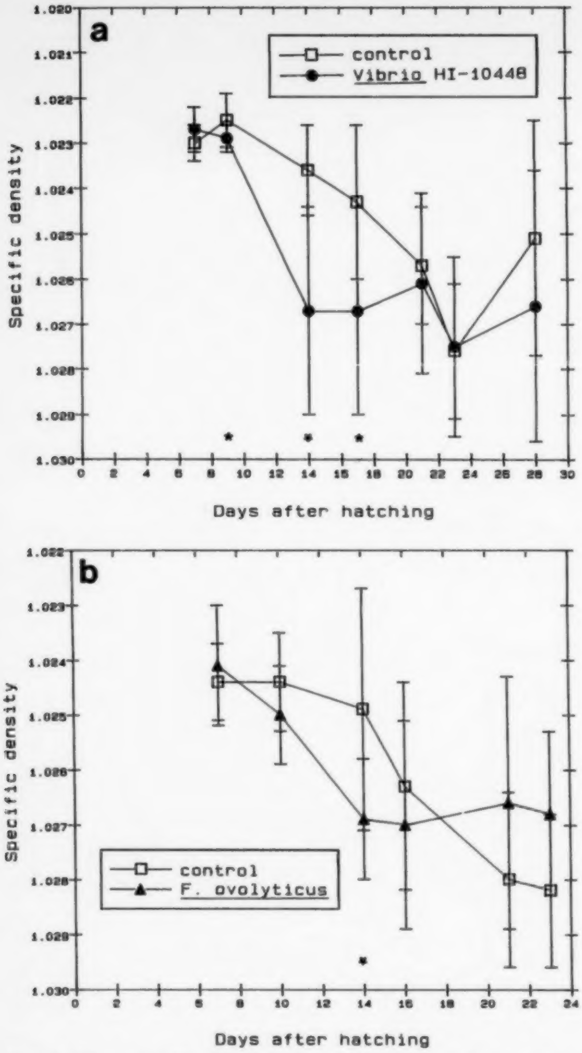

FIG. 5. Mean specific density ($g \cdot cm^{-3}$) of halibut larvae in the experiment with *Vibrio* sp. HI-10448 (a) and with *Flexibacter ovolyticus* (b) and their respective control groups, with standard deviation. The differences were significant at the level of $p < 0.05$ (Student's *t*-test) at the samplings marked with an asterisk.

requirements of fish larvae. This may also have been the case in earlier studies in which the behaviour of these larvae was investigated (Naas and Mangor-Jensen 1990; Pittman et al. 1990b; Van der Meeren, 1991). The level of activity is partially governed by the need to avoid predators and, from time of first-feeding, the need to search for food (Skiftesvik 1992). Thus, changes in the level of activity may alter both the susceptibility of larvae to predation, and their ability to search for and catch food items.

The increased specific density of the infected halibut larvae shows that bacterial infections might also cause alterations in the spatial distribution. Changes in buoyancy cause alterations in the vertical distribution of the larvae. Thus, both the reduced activity and the increased specific density would eventually alter the susceptibility of the larvae to predation in nature. This could constitute an indirect mechanism by which pathogens alter the survival potential of their hosts, in addition to the direct mortality caused by bacterial infection.

The mortality curve for the halibut larvae supports the conclusion of Bergh et al. (1992) that both *F. ovolyticus* and *Vibrio* sp. HI-10448 may cause substantial mortality throughout the yolk sac stage. Compared to the experiments described by Bergh et al. (1992), in which *F. ovolyticus* caused high mortalities prior to hatching, the time from infection until hatching was considerably shorter in the present study, allowing the bacteria less time to proliferate on the egg surface. We found no direct relationship between the dose of *F. ovolyticus* and mortality of the halibut larvae. A rapid decrease in viability, probably caused by bacteriophages, is typical of dense cultures of *F. ovolyticus* (Hansen et al. 1992a), although the factors responsible for lysogenic induction are unknown. Phage-mediated lysis of a dense culture could possibly explain the decreased pathogenicity of the highest concentration of *F. ovolyticus*.

The *Vibrio* sp. HI-11360 strain was pathogenic for turbot larvae. In contrast to the results from the halibut experiments, *F. ovolyticus* caused no significant mortality to turbot larvae, indicating that this bacterium is either nonpathogenic to turbot, or at least less pathogenic to turbot than to halibut. This might be due to the differences in the rearing temperatures of the two species. The only significant differences in behaviour among the groups in the turbot experiment were in the activity measurements and the fraction of active larvae between the groups infected with *F. ovolyticus* and the group infected with *Vibrio* HI-11360. Although the control group could not be significantly separated from the infected groups with respect to behavioural parameters, the differences among the infected groups show that the behaviour of turbot larvae might be affected by exposure to bacteria. It could be hypothesized that a nonpathogenic bacterium, as in this case *F. ovolyticus* may well be, could outcompete opportunistic pathogens present, and thus be beneficial to these larvae compared to the control group.

The interspecific difference in the buoyancy response could be related to the differences in habitat of the two species. Halibut spawn at 300–700 m depth (Haug 1990) and virtually nothing is known about the yolk sac larvae of this species in nature. Turbot larvae occur in the upper 10 m of the water column (Nellen and Hempel 1970; Russel 1976). All measurements of specific density of turbot imply that these larvae are distributed in the uppermost part of the water column. Thus, while small changes in the buoyancy of halibut larvae might cause large vertical movements, this would not necessarily be the case for turbot. In addition, halibut hatch at a primitive ontogenetic stage (Pittman et al. 1990b) compared to turbot (Russel 1976). This may explain the interspecific variation in the susceptibility of the larvae to different pathogens. The mortality in the infected groups of halibut larvae was probably highly selective, as the control groups tended to have higher buoyancy toward the end of the experiment.

Different species of the genus *Vibrio* are known to cause systemic infections in various fish species (Egidius 1987). Ultimately, the bacteria may infiltrate muscular tissue including the heart, as demonstrated by Totland et al. (1988) for Atlantic salmon (*Salmo salar*) infected by *V. salmonicida*. From studies on halibut larvae, infiltration of heart tissue and blood vessels has been shown, as well as the presence of large amounts of bacteria among the gill arches (Bergh et al. 1992). Reduced activity and problems with buoyancy regulation could be viewed as a response to such pathological changes.

To counteract larval sinking and avoid the accumulation of bacteria, large-scale rearing of halibut yolk-sac larvae has so far been based on upwelling incubators of the types described by Pittman et al. (1989, 1990a), although high rates of flow may cause decreased survival and yolk-sac utilization compared to low rates (Opstad and Bergh 1993). The results from our buoyancy measurements show that infected halibut larvae tend to be less buoyant; thus they may sink to the bottom of the incubators, close to the water inlet through which dead larvae are removed. Thus, the inlet water will pass through a zone with dead and infected larvae, a probable source of continuous release of bacteria, as has been shown for Atlantic salmon (*Salmo salar*) smolts infected with *Aeromonas salmonicida* subsp. *salmonicida* (Enger et al. 1992). Consequently, the upwelling water may become contaminated before reaching the healthy larvae. This implies a mechanism by which pathogens, once established in an incubator, might proliferate rapidly.

The reduced activity of the infected halibut larvae occurred early in the experiment, well in advance of the increased mortality. This may provide a means by which infected larvae could be detected at an early stage. In contrast to complicated histological and microbiological techniques, behavioural responses are relatively easy to recognize. Thus, the measurement of such responses constitutes a possible means by which infected larvae could be sorted out. The increased specific density of the halibut larvae should make it possible to sort out the infected larvae at the bottom of the incubators, thereby reducing the infectious load of pathogens in the incubators.

We conclude that pathogenic bacteria are capable of influencing the activity and positioning in the water column of their hosts. Thus, any investigation of the behaviour of larval fish should take into account the potential effects of bacterial infection.

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Spatial Variation in Food-Limited Growth of Juvenile Greenback Flounder, *Rhombosolea tapirina*: Evidence from Otolith Daily Increments and Otolith Scaling

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Jenkins, G.P., M. Shaw, and B.D. Stewart. 1993. Spatial variation in food-limited growth of juvenile greenback flounder, *Rhombosolea tapirina*: evidence from otolith daily increments and otolith scaling. Can. J. Fish. Aquat. Sci. 50: 2558–2567.

Growth rates of juvenile flounder, *Rhombosolea tapirina*, determined from daily increment number, and the relationship between otolith and fish sizes (otolith scaling), were compared between two adjacent areas. Swan Bay, Victoria, a sheltered bay with a well-developed seagrass-detrital system, supports higher populations of prey and feeding rates of juvenile flounder than Port Phillip Bay, an area more exposed to waves and tidal currents. Temperature was significantly higher in Swan Bay (though generally less than 1°C). Growth rates determined from daily increment number were similar within bays, but significantly different between bays. The pooled growth rate for Swan Bay (0.29 mm · d^{-1}) was significantly higher than for Port Phillip Bay (0.17 mm · d^{-1}). The same pattern was found for otolith scaling. Most of the variation in growth rates between the two bays was apparently related to food supply. A laboratory experiment indicated that otolith growth rate had a minimum level which was independent of somatic growth rate, and an additional component which was highly correlated with somatic growth rate. This resulted in an exponential decrease in otolith growth per unit somatic growth with increasing somatic growth rate such that variation in otolith scaling would be greatest at low growth rates.

On a comparé, entre deux secteurs contigüs, les taux de croissance de plies juvéniles, *Rhombosolea tapirina* , à partir de leur progression quotidienne, avec le rapport qui existe entre l'otolithe et la taille des poissons (mesure comparée des otolithes). La baie Swan, Victoria, est une baie abritée, constituée d'un système bien développé d'algues marines et de détritivores, qui loge de plus grandes populations de proies et permet un taux d'alimentation des plies juvéniles supérieur à celui de la baie Port Phillip, un secteur qui est davantage exposé aux vagues et aux courants de marée. La température de l'eau dans la baie Swan était significativement supérieure (quoique l'écart ne dépassait généralement pas 1°C). Les taux de croissance déterminés par la progression quotidienne étaient semblables à l'intérieur de chacune des baies, mais significativement différents entre les baies. Le taux de croissance totalisé dans la baie Swan (0,29 mm · d^{-1}) était significativement supérieur à celui de la baie Port Phillip (0,17 mm · d^{-1}). Apparemment, les écarts dans les taux de croissance mesurés dans les deux baies, sont en rapport avec l'abondance des proies. Il en allait de même pour la mesure des otolithes. Une expérience en laboratoire a indiqué que le taux de croissance des otolithes a un seuil qui est indépendant du taux de croissance somatique, et qu'il comporte un autre élément qui est en étroite corrélation avec le taux de croissance somatique. Cela a donné une diminution exponentielle de la croissance des otolithes en fonction de la croissance somatique, à mesure que la croissance somatique progressait, de manière à ce que les variations de la mesure comparée des otolithes seraient les plus considérables à de faibles taux de croissance.

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Growth rate may be a critical factor in the survival of larval and juvenile fish because it influences the duration of the period of susceptibility to size-selective predation (Smith 1985). Growth rates can strongly influence survival during periods of intense predation pressure, favouring the survival of faster-growing individuals (Post and Prankevicius 1987). Even if growth rate does not influence mortality, high growth rates will have a positive influence on the production rate of young fish from a given area.

Two of the most important influences on growth rate of juvenile fish are temperature and food supply (Weatherly and Gill 1987). Temperature would be expected to have the major influence on growth rates except in situations where food is limiting. Juvenile flatfish often occur in "nursery" areas where conditions for survival of juveniles are thought to be enhanced (Rosenberg 1982). Spatial variation in growth rates of juvenile flatfish has usually (Zijlstra et al. 1982; Poxton et al. 1983; Bergman et al. 1987; Karakiri et al. 1989; Sogard and Able 1992), but not always (Rosenberg 1982), been found. Spatial variation in growth rates of juvenile flatfish has been attributed to food limitation (Poxton et al. 1983; Bergman et al. 1987; Karakiri et al. 1989) or temperature only (Kuipers 1977; Zijlstra et al. 1982; van der Veer et al. 1990, 1991).

The greenback flounder, *Rhombosolea tapirina*, is a commercially important pleuronectid in southern Australia (Hutchins and Swainston 1986). Metamorphosing-larvae immigrate to shallow, unvegetated habitats, which are utilised in the early-juvenile stage (Crawford 1984; May and Jenkins 1992). Otoliths of juvenile *R. tapirina* contain daily increments (Stewart and Jenkins 1991) which can be used to determine size-at-age (May and Jenkins 1992). The relationship between size and age can therefore be used to examine spatial variation in growth rate. Furthermore, there is a strong relationship between otolith size and fish size in this species (May and Jenkins 1992). The relationship between otolith size and fish size, or otolith scaling (sensu Secor and Dean 1989), has been found to vary with growth rate in some species; with slower-growing populations having relatively larger otoliths (Reznick et al. 1989; Secor and Dean 1989; Wright et al. 1990). It follows that variation in otolith scaling could be used to examine variation in somatic growth rate among populations (Reznick et al. 1989; Secor and Dean 1989).

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In the present study, we examine spatial variation in the growth rate of juvenile *Rhombosolea tapirina* from Swan Bay and adjacent areas of Port Phillip Bay, Victoria (Fig. 1). Sediments are finer and organic content of sediment is higher in Swan Bay (Shaw and Jenkins 1992). Moreover, prey abundances and feeding rates of juvenile *R. tapirina* were significantly higher in Swan Bay (Shaw and Jenkins 1992). We hypothesized that these differences in prey production would lead to significantly higher growth rates in Swan Bay compared with Port Phillip Bay. We used otoliths, from juvenile *R. tapirina* which had previously been examined for feeding rates by Shaw and Jenkins (1992), to examine variation in growth rates at the population level, as indicated by size at age, and otolith scaling. We also describe a laboratory experiment to elucidate the underlying relationship between otolith growth rate and somatic growth rate which results in the somatic growth rate effect on otolith scaling.

Materials and Methods

Sampling was conducted at three sites within Swan Bay and three sites on the adjacent coast of Port Phillip Bay over the period 1 August 1990 to 6 September 1990 (Fig. 1). All six sites were unvegetated but adjacent to seagrass beds. Field sampling methods for collecting juvenile flounder are described by Shaw and Jenkins (1992).

The water temperature was measured to the nearest 0.1°C at all sites. Measurements were taken at fortnightly intervals from 17 May to 20 August 1990. Water temperature was measured at the level of the substrate (approximately 0.25 m). All measurements were taken at approximately mid tide within 1 h on a sampling day to reduce the effects of variation in tide and weather.

Laboratory Analyses

The standard length (SL = distance between the tip of the snout and the posterior edge of the caudal peduncle) of each flounder was measured to the nearest 0.1 mm with the aid of a dissecting microscope fitted with an ocular micrometer. After removal of gut contents (Shaw and Jenkins 1992), specimens were placed in a drop of water on a microscope slide and the utricular (lapilli) and saccular (sagittae) otoliths were dissected out using electrolytically-sharpened tungsten probes. Contrast between the otoliths and surrounding tissue was maximised by using a polarizing light source fitted to the stereo microscope. The otoliths were left to dry, flat side up, and then were covered with a drop of immersion oil.

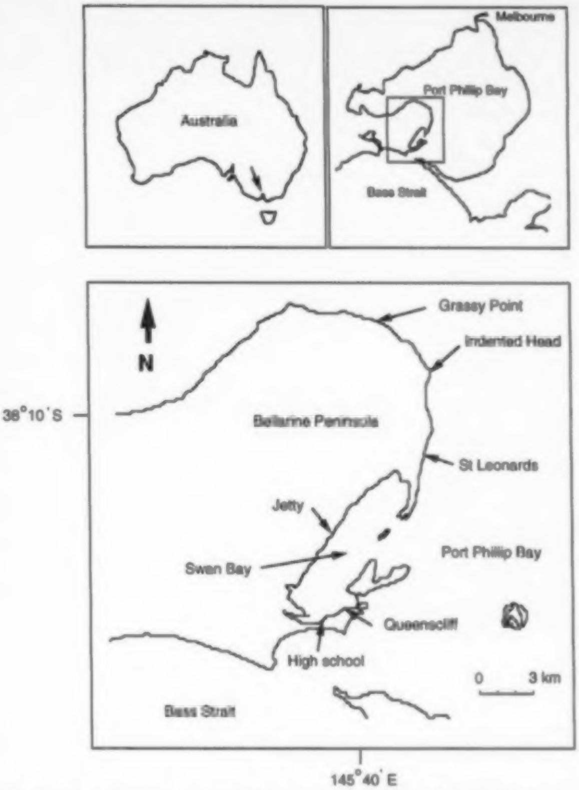

FIG. 1. Location of sampling sites in Swan Bay and Port Phillip Bay, Victoria, from which juvenile flounder were collected. *Insets*: Location of Swan Bay in Port Phillip Bay and location of Port Phillip Bay on the Australian coast.

Otolith measurements and counts were made with a compound microscope. A video camera connected to the microscope produced an image which was displayed and digitised on a Commodore "Amiga" computer. Specimens were examined randomly and blindly by two people. We attempted to count the post-settlement increments on all four otoliths. Post-settlement increments were defined as those deposited after the transition zone of poorly defined increments which forms over the period of metamorphosis and settlement (May and Jenkins 1992). A clarity value for increments was assigned in the range of 1–4; 1 representing low clarity and 4 representing the clearest. Readings having a clarity of 2 or greater were accepted. When the mean increment count determined by independent readings differed by more than two the data were discarded. Only two specimens were rejected by these criteria. Because body size is more strongly related to sagittal radius than to lapillus radius in juvenile *R. tapirina* (Thompson 1990), only growth rate of sagittae was examined in this study. Moreover, lapilli showed distinct bilateral asymmetry over the size range examined (Thompson 1990; Sogard 1991). Each sagitta was measured along the line of maximum otolith radius, which occurred on the postrostrum.

Laboratory Experiment

Juvenile flounder were collected, using the methods described

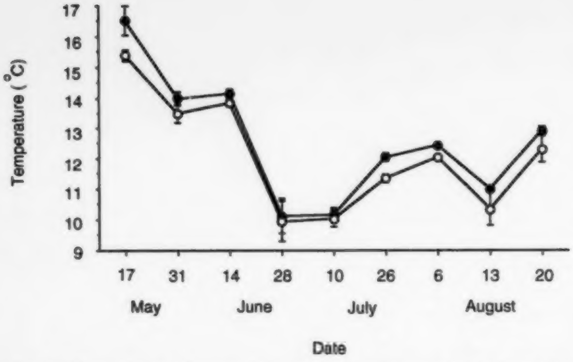

FIG. 2. Time course of mean water temperature from sites in Swan Bay and Port Phillip Bay. Closed circles, Swan Bay; open circles, Port Phillip Bay. Error bars = S.D.

by Shaw and Jenkins (1992), at Grassy Point (Fig. 1) on 24 September 1991. Live flounder were transferred to a 20-L container with aeration, and transported to the laboratory. Flounder were placed in an aerated 20-L aquarium and fed *Artemia* nauplii ad libitum. Two days acclimation was allowed before flounders were transferred to another aquarium containing 400 mg · L^{-1} oxytetracycline hydrochloride for 24 h. After treatment, flounder were anaesthetised with benzocaine and the SL was measured. Individual flounder were then transferred to containers of approximately 2-L volume which were placed in a flow through water table connected to a recirculating seawater system. An opening at each end of the container covered with 35 μm nylon mesh allowed exchange with the water table.

Two feeding treatments were used, a "high" treatment where flounder were fed ad libitum with *Artemia* nauplii every second day, and a "low" treatment where flounder were fed ad libitum with *Artemia* nauplii once per week. Ten juvenile flounder of a similar range of sizes were assigned to each treatment. Containers from each treatment were randomly placed in the water table. Flounder were exposed to natural light entering windows in the aquarium building. The experiment was run for 32 d. Fish were in good condition at the end of the experiment. Daily water temperature ranged from 12.7 to 18.2°C. At the end of the experiment, flounders were again anaesthetised with benzocaine and measured before preservation in 95% ethanol.

Otoliths were removed and one sagitta from each fish was ground and polished. Sagittae were cleaned with alcohol and then mounted, flat side up, in epoxy resin. Otoliths were manually ground with a succession of 15–12 μm wet and dry sandpaper until the otolith surface was reached. They were then polished with 6 μm diamond paste on a lapping machine. The lapping machine ensured that otoliths were polished on a horizontal plane. Whole and ground sagittae from each fish were observed under an incident fluorescence microscope with excitation bands from 450 to 490 nm and barrier filters at 510 and 515 nm to examine fluorescence produced by tetracycline incorporation. Otoliths were also examined using normal transmitted light passed through a single polarising filter. A magnification of 1250× was used in both cases. The position of the tetracycline mark relative to the sagitta edge was determined by alternately viewing the specimen under UV and normal transmitted light. The radius from this position to the sagitta edge along the postrostral axis was measured. A random selection of sagittae was also examined with a scanning electron microscope (SEM). Ground and polished sagittae were etched with 0.02 HCl for 30–45 s and then coated with gold using a sputter coater for 2.5 min.

TABLE 1. Univariate repeated measures analysis of variance of temperature variation between bays with Greenhouse-Geisser conservative *F*-test applied. $^{*}P < 0.05$, $^{**}P < 0.001$, ns = not significant.

Source	df	MS	*F*
Bay	1	3.48	39.27 *
Site	4	0.09	
Date	8	23.47	238.18 **
Bay × date	8	0.14	1.45 ns
Error	32	0.10	

Data Analysis

Temperature differences between bays and among sites within bays were compared over time using a univariate repeated measures analysis of variance. The Greenhouse–Geisser conservative *F*-test was applied. Variation in size at age was analysed using a nested analysis of covariance with post-settlement increment number as the covariate. Variation in otolith scaling was analysed using a nested analysis of covariance with otolith radius as the covariate. In all analyses, bays were considered to be a fixed, main effect and sites within bays were random effects. All statistical analyses were conducted using the 'SYSTAT' computer programs (Wilkinson et al. 1992). Assumptions for parametric analyses were examined using box plots and the distribution of residuals.

Results

Temperature

Water temperature fell rapidly until late June, early July and then increased slowly (Fig. 2). Water temperatures were significantly higher, on average approximately 0.5°C, in Swan Bay (Table 1), and were between 0.5°C and 1°C higher in this bay during August when juvenile flounder were collected (Fig. 2).

Size at Age

Sufficient numbers of juvenile *Rhombosolea tapirina* for analysis were collected at Jetty and Queenscliff in Swan Bay, and Grassy Point and Indented Head in Port Phillip Bay (Shaw and Jenkins 1992). Linear regressions between size and post-settlement increment number were highly significant at sites in Port Phillip Bay and Swan Bay (Table 2). Growth rates at sites within each bay were similar. Nested analysis of covariance showed a significant interaction between bay and increment number, but not between site and increment number, indicating that growth rates were significantly different between bays but not between sites within bays (Table 3). The pooled regression for Swan Bay ($n = 98$, $F = 425.34$, $P < 0.001$) indicated a growth rate of 0.29 mm · d^{-1}, which was approximately 70% higher than the growth rate of 0.17 mm · d^{-1} indicated by the pooled regression for Port Phillip Bay ($n = 73$, $F = 157.94$, $P < 0.001$) (Fig. 3).

TABLE 2. Regression equations relating standard length to post-settlement increment number, and otolith radius, for juvenile flounder from sites within Swan Bay and Port Phillip Bay. $^{*}P < 0.05$, $^{**}P < 0.001$, ns = not significant.

Site	Equation	*n*	R^2	*F*
Post-settlement increment number				
Port Phillip Bay				
Grassy Point	$SL = 0.16x + 4.94$	54	0.68	110.59**
Indented Head	$SL = 0.18x + 4.58$	19	0.70	40.21**
Swan Bay				
Jetty	$SL = 0.30x + 4.34$	50	0.86	288.50**
Queenscliff	$SL = 0.29x + 4.22$	48	0.73	134.84**
Otolith radius				
Port Phillip Bay				
Grassy Point	$SL = 0.069x + 0.36$	46	0.81	182.13**
Indented Head	$SL = 0.069x + 0.06$	20	0.86	114.17**
Swan Bay				
Jetty	$SL = 0.085x - 0.74$	49	0.92	515.42**
Queenscliff	$SL = 0.080x - 0.05$	48	0.89	359.71**

TABLE 3. Analysis of covariance of standard length and post-settlement increment number for juvenile flounder collected in Port Phillip Bay and Swan Bay with two sites nested within each bay. $^{*}P < 0.05$, $^{**}P < 0.001$, ns = not significant.

Source	df	MS	*F*
Bay	1	0.79	6.09 ns
Site (bay)	2	0.13	0.14 ns
Increment no.	1	423.77	464.96 **
Bay × incr. no.	1	29.66	165.51*
Site (bay) × incr. no.	2	0.18	0.20 ns
Error	163	0.91	

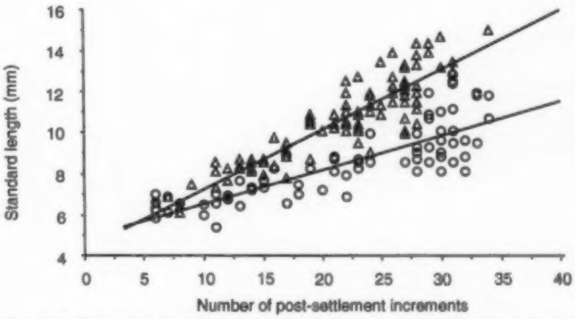

FIG. 3. Relationship between post-settlement increment number and size of juvenile flounder, data pooled within bays, with fitted regression lines. Open circles, Port Phillip Bay; $SL = 0.17x + 4.85$, $R^2 = 0.69$; closed triangles, Swan Bay; $SL = 0.29x + 4.35$, $R^2 = 0.82$.

Otolith Scaling

Linear regressions between SL and otolith radius were highly significant at sites in Port Phillip Bay and Swan Bay (Table 2). Regression slopes at sites within each bay were similar. Nested analysis of covariance showed a significant interaction between bay and otolith radius, but not between site and otolith radius, indicating that otolith scaling was significantly different between bays but not between sites within bays (Table 4). The pooled regression for Swan Bay ($n = 96$, $F = 921.03$, $P < 0.001$) indicated a regression slope of $0.083\ \mathrm{mm} \cdot \mu\mathrm{m}^{-1}$, which was approximately 20% higher than the regression slope of $0.068\ \mathrm{mm} \cdot \mu\mathrm{m}^{-1}$ indicated by the pooled regression for Port Phillip Bay ($n = 66$, $F = 308.23$, $P < 0.001$) (Fig. 4).

Laboratory Experiment

Tetracycline was incorporated into all otoliths examined, at a point corresponding to a change to a more translucent crystalline structure with low increment contrast and clarity (Fig. 5), and often a dark "check" mark. Somatic and otolith growth rate was higher in the high food treatment (Table 5), with the increase in log-transformed SL significantly higher in the high food treatment (ANOVA df = 1/18, $F = 88.604$, $P < 0.001$), as was the increase in log-transformed otolith radius (ANOVA df = 1/17, $F = 50.078$, $P < 0.001$). Clear increments were only present in the fastest-growing fish ($>2.0\ \mathrm{mm} \cdot \mathrm{d}^{-1}$) and the number approximated the period of the experiment; however, increments in sagittae from slower-growing individuals were extremely narrow, of poor contrast, and could not be reliably counted. Examination with SEM improved resolution only slightly (Fig. 6), and increments from all but the fastest-growing fish were still difficult to interpret.

There was a highly significant linear regression between otolith growth rate and somatic growth rate (df = 1/16, $F = 202.39$, $P < 0.001$; Fig. 7):

$$\text{(1)}\quad \text{Otolith growth rate} = 10.81 \times \text{somatic growth rate} + 0.42 \qquad R^2 = 0.93.$$

The intercept of this equation indicates that the sagittae grew by an average of approximately 0.4 μm even when somatic growth rate was near zero. Dividing somatic growth rate by otolith growth rate for data points and the regression line shown in Fig. 7 gave an estimate of otolith scaling. When otolith scaling is plotted against absolute somatic growth rate, there is an initial rapid increase which tends to level out at higher growth rates (Fig. 8). One specimen from the slow-growth treatment showed

TABLE 4. Analysis of covariance of standard length and otolith radius for juvenile flounder collected in Port Phillip Bay and Swan Bay with two sites nested within each bay. $^*P < 0.05$, $^{**}P < 0.001$, ns = not significant.

Source	df	MS	F
Bay	1	0.43	2.32 ns
Site (bay)	2	0.19	0.40 ns
Radius	1	474.03	1006.75 **
Bay × radius	1	3.85	22.31 *
Site (bay) × radius	2	0.17	0.37 ns
Error	155	0.47	

atypically high otolith scaling (Fig. 8). This resulted from a less than expected increase in otolith size, probably due to uneven growth at the otolith edge, or misinterpretation of position of the tetracycline mark.

Discussion

Field Data

Temperature can exert a major control over growth rate and ingestion rates of fish (Brett 1979), including juvenile flatfishes (Malloy and Targett 1991). However, the small temperature difference recorded between bays in this study would seem insufficient to explain the large difference in growth rate observed. For example, an optimal growth rate equation has been derived in the laboratory for flounder, *Platichthys flesus*, by Glazenburg (1983) (cited in van der Veer et al. 1991):

$$\Delta L = 1.2T - 7.2\ (\mathrm{mm} \cdot \mathrm{mo}^{-1})$$

where ΔL = length increase (millimetres per month) and T = mean water temperature (degrees Celsius). If this equation is applied to growth rate of juvenile *Rhombosolea tapirina* at temperatures of 11 and 12°C, approximating temperatures in Port Phillip and Swan Bays in August, growth rates of approximately 2.0 and 2.4 $\mathrm{mm} \cdot \mathrm{d}^{-1}$ result, a difference of 17%. Thus, temperature difference would appear to only partly explain the observed difference in growth rates between the two bays.

The second major determinant of growth rate is food supply (Weatherly and Gill 1987). Abundances of potential prey for juvenile *R. tapirina* are higher in Swan Bay, and this leads to an 80% higher ingestion rate (Shaw and Jenkins 1992). This difference in ingestion rate appeared to have a direct influence on growth rates, and was probably of greater importance than temperature in causing the 70% higher growth rate in Swan Bay compared with Port Phillip Bay.

The observation that spatial variation in growth rate was apparently mainly caused by variation in food supply is consistent with a number of studies on juvenile flatfish. Poxton et al. (1983) found differing mean lengths at the end of the summer growth-season for juvenile plaice, *Pleuronectes platessa*, in three bays and suggested that this was related to the influence of benthic productivity on growth rate. Bergman et al. (1987) found a strong correlation between mean length of plaice at the end of the growing season and abundance of macrobenthos in the Wadden Sea. Both of these studies rely on the assumption that differences in mean length equate to differences in growth rate. In contrast, Karakiri et al. (1989) examined otolith daily increments in juvenile plaice and found significant differences in size-at-age and increment widths. Differences were attributed to food supply because temperature conditions were relatively constant. Our study, together with data from the same fish suggesting food limitation based on prey abundances and feeding rates (May and Jenkins 1992), provides strong evidence for food limited growth. In contrast to these results, comparison of growth rate of juvenile flatfish with models based on optimal growth rate in the laboratory has generally indicated that growth rate is limited by temperature only (Zijlstra et al. 1982; van der Veer 1986; van der Veer et al. 1990, 1991).

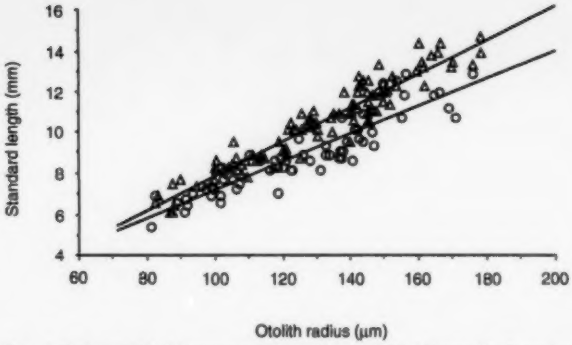

FIG. 4. Relationship between otolith radius and SL of juvenile flounder, data pooled within bays, with fitted regression lines. Open circle, Port Phillip Bay; $\mathrm{SL} = 0.068x + 0.33$, $R^2 = 0.83$; closed triangle, Swan Bay; $\mathrm{SL} = 0.083x - 0.48$, $R^2 = 0.91$.

In general, Swan Bay produces and retains large quantities of macrophyte detritus, leading to organically-enriched sediments and high abundances of potential prey for juvenile flounder relative to Port Phillip Bay (Shaw and Jenkins 1992). This productivity, in turn, leads to higher growth rates of young fish. Faster growth rate may in turn lead to a reduction in size-selective mortality (Smith 1985; Post and Prankevicius 1987). Therefore, Swan Bay is an example of a "nursery" area (Rosenberg 1982) which provides conditions of enhanced growth rate and possibly survival of *R. tapirina*.

Other factors may have contributed to differences in growth rates between bays but are unlikely to have been of major importance. Although tidal currents were greater in Port Phillip Bay, burying behaviour of juvenile flounder (Gibson and Robb 1992) and boundary layer effects would minimise impact. Greater wave action in Port Phillip Bay may be more important, displacing flounder from the sediment and requiring expenditure of energy to maintain position, although burying behaviour would again reduce the effect (Gibson and Robb 1992).

In addition to differences in size-at-age, otolith scaling varied significantly between bays, providing further evidence for differences in growth rates. The presence of relatively larger otoliths in slower-growing fish has been demonstrated for a number of species (Reznick et al. 1989; Secor and Dean 1989; Wright et al. 1990), including larval flatfish (Hovenkamp 1990). The advantage of using otolith scaling as a relative measure of growth rate is that sample processing is much faster than when counting increments, and when clear daily increments are not present, it provides an alternative method.

Laboratory Experiment

There was a change in increment contrast and clarity, and greater transparency of the otolith in general, for otolith material

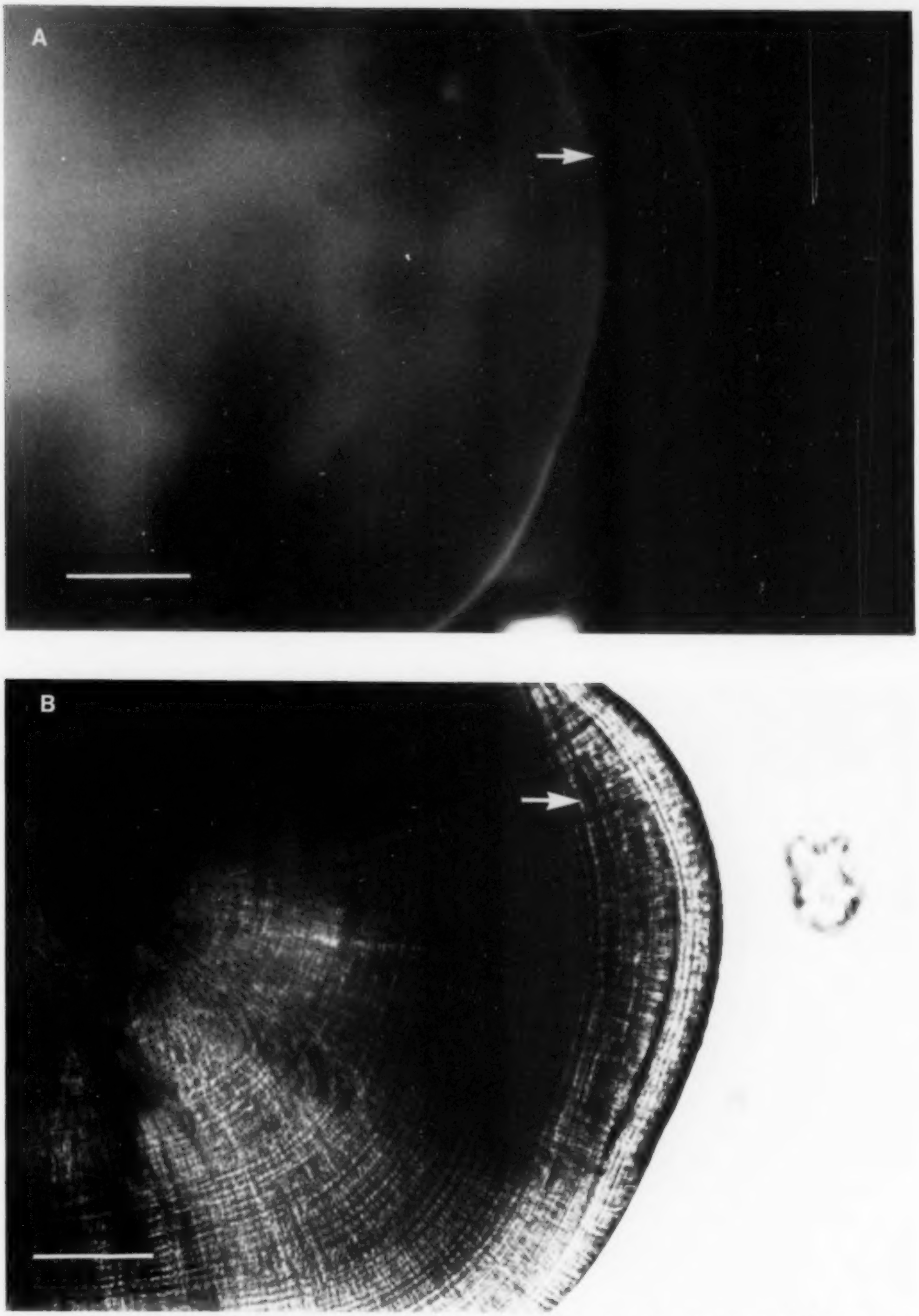

FIG. 5. Light micrographs of the postrostrum of a sagitta from a juvenile *Rhombosolea tapirina* treated with tetracycline and maintained in the laboratory for 32 d. (A) Incident UV light, arrow indicates point of tetracycline incorporation. (B) Transmitted incandescent light, arrow indicates change in increment clarity and contrast. Scale bar = 60 μm.

TABLE 5. Somatic and otolith growth rate of juvenile flounder in high and low food treatments.

	Food					
	High			Low		
	$\bar{X}$	SE	Range	$\bar{X}$	SE	Range
Standard length (μm)						
Initial	18.4	1.17	10.9–24.3	20.2	1.16	12.5–24.5
Final	23.3	0.99	18.6–27.7	21.0	1.15	13.3–24.9
Δ	4.9	0.53	1.8–8.5	0.83	0.12	0.4–1.5
Otolith radius (μm)						
Initial	278	17.2	197–352	267	17.5	166–353
Final	341	17.6	266–403	287	17.0	189–365
Δ	68	6.7	34–106	12	2.5	12–37

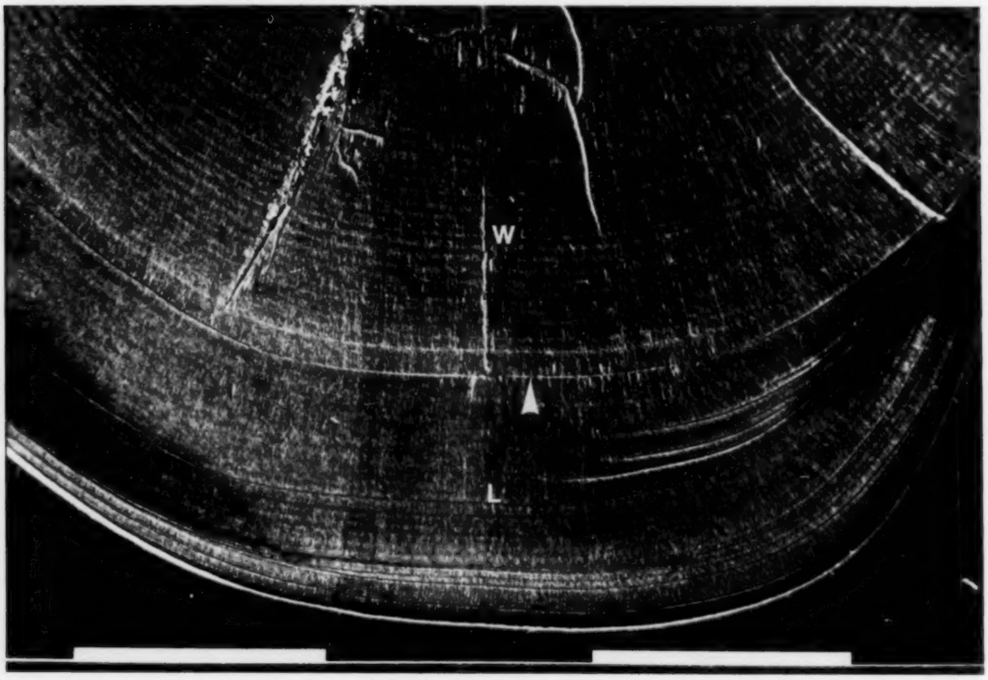

FIG. 6. Scanning electron micrograph of the postrostrum of a sagitta from a juvenile *Rhombosolea tapirina* treated with tetracycline and maintained in the laboratory for 32 d. 'W' denotes portion of otolith formed before collection with clear daily increments, "L" denotes portion of otolith formed in the laboratory. Arrow indicates check mark. Scale bars = 100 μm.

laid down in the laboratory compared with that previously formed in the field. This change in otolith microstructure is common for many species held in the laboratory (Geffen 1982; Campana 1983; Neilson and Geen 1985) including juvenile plaice, *Pleuronectes platessa* (Alhossaini and Pitcher 1988). Tetracycline treatment in our study apparently also caused prominent checks in the otolith, similar to those observed in sagittae of plaice (Alhossaini and Pitcher 1988), and winter flounder, *Pseudopleuronectes americanus* (Sogard 1991). These results support the assumption by Stewart and Jenkins (1991) that change in increment contrast and clarity and the presence of prominent check marks in sagittae of *R. tapirina* corresponded to transfer to the laboratory and tetracycline treatment. In the study by Stewart and Jenkins (1991), the concentration of tetracycline used (250 mg · L^{-1}) was apparently not sufficient to mark otoliths.

We could not reliably count daily increments in slow-growing flounder in the laboratory. This adds to the accumulating evidence that the accuracy of daily increment counts for slow-growing fish is limited by the resolving power of light microscopes, and problems are compounded for laboratory-reared fish where increments are often indistinct using light microscope or

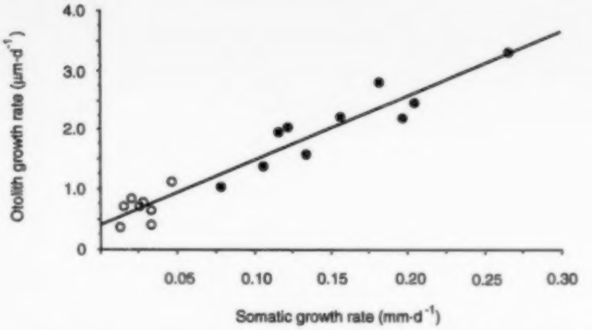

FIG. 7. The relationship between average somatic and otolith growth rate of juvenile *Rhombosolea tapirina* maintained in the laboratory for 32 d. Open circles, low-food treatment; closed circles, high-food treatment.

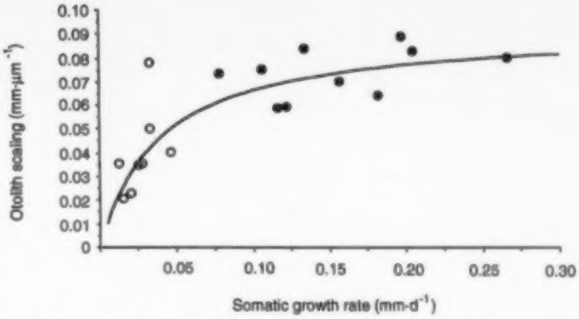

FIG. 8. The relationship between otolith scaling (somatic growth rate per unit of otolith growth rate) and somatic growth rate for juvenile *Rhombosolea tapirina* maintained in the laboratory for 32 d. Open circles, low-food treatment; closed circles, high-food treatment.

SEM (Campana et al. 1987; Jones and Brothers 1987; Alhossaini and Pitcher 1988; Bailey and Stehr 1988). Field collected specimens tend to have greater clarity and contrast of daily increments than laboratory-reared specimens. For example, we routinely measure increments down to 1.0 µm width using light microscopy and SEM on otoliths from field-collected *R. tapirina* juveniles (May and Jenkins 1992).

In our study there was a highly significant linear-relationship between otolith and somatic growth rate; however, there appeared to be minimum deposition on the otolith averaging 0.4 µm that was independent of growth rate (it occurred when growth rate was near zero). This minimum deposition (or age effect) resulted in otolith scaling which was dependent on somatic growth rate. Simple calculations can be used to examine the extent to which the laboratory determined relationship between otolith growth rate and somatic growth rate can explain differences in otolith scaling observed in the field. If 10 mm SL flounder are assumed to have grown at a constant rate of 0.17 mm · d^{-1} (the Port Phillip Bay average) and 0.29 mm · d^{-1} (the Swan Bay average) then individuals would be 59 and 34 d of age, respectively. Application of the laboratory derived equation to each day of growth rate results in predicted otolith sizes of 132.8 µm for Port Phillip Bay and 122.6 µm for Swan Bay, a difference of 8%. This difference was entirely due to the accumulating "age effect" over time. The average otolith size based on field data were 142.2 µm for Port Phillip Bay and 126.27 µm for Swan Bay, a difference of 12.6%.

It would be unreasonable to assume that the laboratory equation could be used to accurately estimate the extent of otolith scaling in the field. For example, it must be assumed that the relationship between otolith growth rate and somatic growth rate derived in the laboratory experiment was the same in the smaller field-collected specimens. This is not necessarily the case, as developmental changes in this relationship can occur. In the case of *R. tapirina*, the rate of otolith growth rate compared to somatic growth rate is much slower in the larval stage relative to the juvenile stage, even though the somatic growth rate is also slower (Jenkins 1987; May and Jenkins 1992). During metamorphosis there is an inflection in the relationship where otolith growth rate continues while somatic growth rate is apparently minimal (May and Jenkins 1992). The relationship may change in the juvenile stage as a consequence of changing otolith shape (Brothers and McFarland 1981; Fowler 1989).

The laboratory study does not take into account possible temperature effects on otolith scaling. Otolith growth rate can uncouple from somatic growth rate at superoptimal temperatures (Mosegaard et al. 1988). Although experimental fish were kept under the same temperature regime, field collected specimens came from areas with different temperature regimes. May and Jenkins (1992) found that otolith growth rate was highly correlated with temperature in Swan Bay, but somatic growth rate may not have shown an equal response at high temperatures, leading to "uncoupling." Further experiments investigating the interaction between temperature and the "age effect" would be useful.

Otolith scaling showed an initial rapid increase and then levelled off with increasing growth rate because the time taken to grow a specific amount (the "age effect") decreases exponentially with increasing growth rate. Therefore, comparisons of otolith scaling involving slow-growth rate populations will be more sensitive than those involving fast-growth rate populations. For example, a population of 10 mm flounder growing at a constant rate of 0.05 mm · d^{-1} would be 200 d of age and have an otolith size of 192.1 µm. If the growth rate was doubled to 0.1 mm · d^{-1} then the population would be 100 d of age and have an otolith size of 150.1 µm, a difference of 28%. However, if the comparison is made between a population with a growth rate of 0.2 mm · d^{-1} doubling to 0.4 mm · d^{-1}, we find that a population of 10 mm flounder growing at 0.2 mm · d^{-1} would be 50 d of age and have an otolith radius of 129.1 µm, compared with 118.6 µm for a 25 d old population growing at 0.4 mm · d^{-1}, a difference of 9%. The larger variation in otolith scaling for the slower growing populations is due to the greater difference in age (100 d) compared to the faster growing populations (25 d).

Variability in growth rate, however, will not affect scaling relationships between populations of similar age. For example, if a population of flounder of 10 mm length and a constant growth rate of 0.2 mm · d^{-1} is compared to a population of the same length which alternates between 0.1 and 0.3 mm · d^{-1} on a daily basis, both populations would have an otolith radius of 129.1 µm, because both populations would be 50 d of age.

A significant linear relationship between otolith growth rate and somatic growth rate for juvenile flounder was also obtained by Alhossaini and Pitcher (1988) and Sogard (1991). In these studies, although the relationship showed greater variability, there was also significant otolith growth rate when somatic growth rate was zero (or negative). Winter flounder in some cases showed no otolith growth rate along the measurement axis, however, growth rate did occur in other parts of the otolith (Sogard 1991).

Observations on the relationship between somatic growth rate and otolith growth rate in juvenile *R. tapirina* are consistent with the DIP model proposed by Secor and Dean (1989, 1992). The model suggests that there is a minimum deposition component of otolith growth rate which is independent of somatic growth rate and there is a second component of otolith growth rate which is correlated with somatic growth rate. The underlying mechanism for the component of otolith growth rate which is independent of somatic growth rate is controversial, with the implication of both an endogenous rhythm of calcification and matrix deposition under genetic and endocrinological control (Secor and Dean 1992), and growth independent metabolism (Mosegaard et al. 1988; Wright et al. 1990). The results of our study do not clarify the relative importance of these factors.

The strong linear-relationship between somatic growth rate and otolith growth rate suggests that back-calculation of fish length is viable. However, the strong relationship between otolith growth rate and somatic growth rate over the period of the experiment (32 d) may not be evident at shorter time scales, particularly at the daily level. Bradford and Geen (1987) found that a strong relationship between otolith growth rate and somatic growth rate over the longer term (51 d) was not evident over shorter periods. In general, otolith growth rate appears to be more conservative than somatic growth rate on a daily scale (Bradford and Geen 1987; Secor and Dean 1992). If back-calculation is attempted, our results support the use of age as a factor in regression-based back-calculation methods to compensate for the "age effect" in otolith scaling (Secor and Dean 1992).

In conclusion, spatial variation in abundances of prey and ingestion rates of juvenile flounder, *R. tapirina*, was directly translated into spatial variability in growth rate, determined from both size-at-age and variation in otolith scaling. A laboratory study on the relationship between otolith and somatic growth rate showed that otolith growth rate had a minimal level which is independent of growth rate, and an additional component which is highly correlated with somatic growth rate. This resulted in an initial rapid increase in otolith scaling followed by a levelling off with increasing somatic growth rate such that variation in otolith scaling is greatest at low growth rates.

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Biochemical Indicators of Oxidative Stress in Fish from Polluted Littoral Areas

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Several biochemical parameters have been analyzed in the livers of fish from south Atlantic Spanish littoral areas exhibiting different pollution levels. *Mugil* sp. (grey mullet) from the Huelva Estuary contained higher concentrations of metals, such as Fe and Cu, than those from a reference area (Cádiz Bay). Similarly, sediments from Huelva showed high concentrations of polyaromatic hydrocarbons, polychlorinated biphenyls, and pesticides. The contaminated animals were subjected to oxidative stress, as indicated by their oxidized glutathione redox status, but showed low malondialdehyde and lipohydroperoxide levels. A battery of antioxidant enzymes increased simultaneously in contaminated fishes, including glutathione peroxidase, superoxide dismutase, catalase, glucose-6-P dehydrogenase, and glutathione reductase. These data suggest that contaminated fish were well protected from oxidative stress. Highly significant increases were also observed in cytochrome P-450-associated ethoxyresorufin-*O*-deethylase activity, and soluble and membrane-bound glutathione-S-transferase, in response to the high levels of organic xenobiotics. We propose that antioxidant enzymes and glutathione-S-transferase might be useful tools for the biomonitoring of environmental pollution.

On a étudié plusieurs paramètres dans les foies des poissons provenant des zones avec différents niveaux de pollution au long du littoral Sud-Atlantique espagnol. Les individus de *Mugil* sp. provenant de l'estuaire de Huelva contenaient des concentrations en Fe, Cu plus grandes que ceux qui provenaient d'une zone de reference (Cádiz). En plus, les sediments de Huelva contenaient concentrations plus grandes de hidrocarbons polyaromatiques, biphenyl polychlorinées et pesticides. Ces animaux étaient soumis á une situation de stress oxydatif comme il était montré par l'état redox de ses contenus en glutathione; néanmoins, on a trouvé des niveaux plus bas de malondialdehide et de lipohidroperoxides dans les poissons contaminés. On a observé aussi l'augmentation simultanée d'un ensemble d'enzymes antioxidatifs. Cette réponse coordonneé incluait la glutathione peroxidase, superoxide dismutase, catalase, glucose-6-P-dehydrogenase, et la glutathione reductase. Toutes ces donneés suggérent que les poissons contaminées étaient bien protégés contre le stress oxydatif. On a observé aussi des augmentations trés significatives dans l'activité etoxiresorufine-*O*-deetilase, dépendant du citocrome P-450, et dans l'activité glutathione transferase, aussi soluble qu'unie á membranes comme réponse aux niveaux élevés de composées xenobiotiques. Nous proposons que les enzymes antioxidatifs et la glutathione transferase peuvent être très utiles dans le contrôle de la pollution environnementale.

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Physical, chemical, and metabolic processes convert O_2 into reactive oxygen species (ROS), such as superoxide or hydroxyl radicals, or the nonradical species hydrogen peroxide or singlet oxygen. In aquatic organisms, ROS can be generated by the autooxidation of mitochondrial electron transport chain components, microsomal cytochrome P-450 or flavoprotein reductases (Winston 1991; Winston and Di Giulio 1991). Environmental contaminants may also enhance the generation of ROS. Thus, transition metals (Cu, Fe) catalyse the production of hydroxyl radicals through the Fenton reaction, while biphenyls, quinones, and nitroaromatics produce superoxide by redox cycling (Sies 1988; Winston and Di Giulio 1991).

Oxidative damages affect nucleic acids, proteins, lipids and carbohydrates (Sies 1986). Living beings are protected from ROS by several defence mechanisms, including such antioxidant enzymes as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, as well as pentose monophosphate pathway enzymes, such as glucose-6-phosphate dehydrogenase, that regenerate NADPH. Low molecular weight antioxidants, such as GSH and vitamins E and C, act in conjunction with these enzymatic defenses (Sies 1986). Oxidative stress results when the antioxidant defenses are overcome by pro-oxidant forces and ROS are not adequately removed. This can occur when antioxidants are depleted and/or the rate of ROS production surpasses that of enzymatic defenses. Intracellular glutathione occurs mainly (>98%) in the reduced form. GSH has many antioxidant functions, such as the control of the protein redox status and the defence against free-radicals and oxygen toxicity (Kosower and Kosower 1978). Most of these functions oxidise GSH to the disulfide form, GSSG, whose concentration is an index of oxidative stress (Kosower and Kosower 1978; Alonso-Moraga et al. 1987). GSSG accumulation leads to increased formation of protein-glutathione mixed disulfides (Brigelius 1985).

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Biotransformation of organic xenobiotics is carried out by several inducible enzymes, such as Phase I cytochrome P-450 or Phase II glutathione transferase, also involved in the activation of known promutagens (Buhler and Williams 1988). The activity of the mixed-function oxidase system (MFO) has been extensively used as an early warning indicator of exposure to organic pollutants (Stegeman and Lech 1991; Gøksoyr and Förlin 1992). Recently, antioxidant enzymes and other biochemical parameters have also been proposed as bioindicators for environmental impact assessment (Livingstone 1991; Winston and Di Giulio 1991; Rodríguez-Ariza et al. 1992).

Pollution by Fe and Cu coming from the open-air Rio Tinto pyrite mines has been clearly established in the Huelva estuary, in the Andalusian south Atlantic littoral zone (Establier 1972). In addition, putative organic xenobiotics of industrial and agricultural origin should also be present. Increased activities of several antioxidant and detoxifying enzymes have been reported in three bivalve molluscs from Andalusian coasts in response to environmental pollution. Ethanolic extracts from these molluscs contained direct-acting and polar genotoxins of the oxidative type. Animals living in polluted sites contained fewer genotoxins than those in reference areas, with corresponding increases in antioxidant and detoxifying enzymes (Rodríguez-Ariza et al. 1992). The present report studies the antioxidant defences of *Mugil* sp. (grey mullet) from the Huelva estuary in search of new bioindicators for marine pollution. We have determined the levels of several metals and organic contaminants, the activities of a range of antioxidative and detoxifying enzymes, the redox status of glutathione and the contents of malondialdehyde and lipohydroperoxides.

Material and Methods

Animals and Sampling Areas

Mugil sp. (grey mullet) were collected in February 1990. The contaminated animals (1.5 kg average weight) were obtained from the Odiel River marshes in the Huelva Estuary. Reference animals (0.5 kg average weight) were from the Guadalete River marshes in Cádiz Bay. Animals were captured with trammel nets and within 10 min their spinal cords were severed and livers excised, frozen in liquid nitrogen and stored at −80°C until analyzed (<3 mo). Livers were individually ground in a mortar with liquid nitrogen and kept at −80°C until analyzed. Assays were carried out in pools made up of identical weights of 10 different individually ground livers.

Metal Determinations

Liver samples were mineralised at 450°C in an electric oven. Metals were then extracted with HCl (0.1 M final concentration) and determined by atomic absorption flame spectrometry using appropriate standards, in a Perkin-Elmer apparatus, model 380.

Organic Xenobiotic Determinations

Sediments were collected by dredge and then frozen, lyophilized, and sieved through 500-μm nylon mesh. The samples were Soxhlet-extracted in cyclohexane for 16 h, evaporated, redissolved in hexane and treated with H_2SO_4 and sodium metabisulfite. Polychlorinated biphenyls and pesticides were determined by gas chromatography (Hewlett-Packard, model 5890) using HP5 and DB1701 columns and electron capture detection. Polyaromatic hydrocarbons were analyzed by HPLC (Hewlett-Packard) with Hypersyl ODS columns (5 μm and 0.46 × 20 cm). Elution was carried out with a linear gradient, starting with acetonitrile:H_2O (65:35) and finishing after 25 min with acetonitrile. A fluorescence detector was used with 340 nm for excitation and 425 nm for emission wavelength.

Preparation of Crude Extracts and Microsomal Fractions

Ground pooled livers were homogenized in 10 mM potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA (4 mL buffer/g ground liver). The homogenates were then centrifuged at 31 000 × g for 1 h and the supernatants filtered through glass wool to eliminate the lipids. Microsomes were obtained from three intact livers, essentially as described by Borheim et al. (1985). Livers were thawed, weighed, washed in ice-cold 0.96% (w/vol) NaCl, and homogenized (2 mL/g liver) using a Potter-Elvehjem apparatus with teflon pestle in 5 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose and 10 mM GSH. Homogenates were diluted with 3 volumes of ice-cold 1.15% (w/vol) KCl and centrifuged at 9 000 × g for 10 min. Supernatants were decanted and centrifuged at 105 000 × g for 60 min. The pellets were resuspended in 15 mL of 1.15% (w/vol) KCl and resedimented at 105 000 × g for 30 min. All steps were carried out at 4°C. The washed microsomes were resuspended in 0.1 M phosphate buffer (pH 7.4, 0.5 mL/g liver) and stored at −80°C until used.

Biochemical Determinations

Determination of the initial activities of the detoxifying or antioxidative enzymes was carried out as described previously (Rodríguez-Ariza et al. 1992). Glutathione-S-transferase was assayed at 30°C with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH. Glutathione peroxidase was measured at 37°C in the presence of 0.24 U glutathione reductase, 0.15 mM NADPH and 1 mM GSH using 0.15 mM H_2O_2 plus 1 mM sodium azide (Se-dependent) or 2 mM cumene hydroperoxide (total) as electron acceptors. Catalase was determined at 25°C in the presence of 20 mM H_2O_2. Glutathione reductase was assayed at 30°C using 2.5 mM GSSG and 0.12 mM NADPH. Superoxide dismutase was measured at 25°C with 19.2 μM cytochrome *c*, 48 μM xanthine, and 0.2 U xanthine oxidase. Ethoxyresorufine-*O*-deethylase activity was assayed at 30°C with 1 μM of 7-ethoxyresorufine and 0.1 mM NADPH. Glucose-6-phosphate dehydrogenase activity was determined at 30°C according to Decker (1977). Protein determination was made according to Lowry et al. (1951).

Malondialdehyde levels were determined as described by Gutteridge (1984) with minor differences: incubations were

TABLE 1. Metal contents in livers of grey mullet (*Mugil* sp.) living in areas with different pollution levels[a].

Metals	Reference (R)	Contaminated (C)	Ratio C:R	VC(%)
Cu	13.7	51.1	3.7	2.7
Fe	206.3	300.0	1.5	2.1
Mn	38.0	43.6	1.1	2.4
Zn	29.5	25.1	0.9	2.3
Total	287.5	419.8	1.5	

[a] Metal contents are expressed as ppm of fresh weight and represent the mean values of three independent determinations. Reference animals were from Cádiz Bay and contaminated fish from the Huelva Estuary. Variation coefficients [VC = $(\sigma_n/x) \cdot 100$] are expressed in percentages.

made in 3 mL total volume and 2.8% (w/vol) trichloroacetic acid was used instead of HCl. Lipohydroperoxide levels were determined following the oxidation of 2-hydroxynaftaldehyde thiosemicarbazone by hydroperoxides according to the kinetic-fluorimetric method of Peinado et al. (1986). The different forms of glutathione, reduced, oxidized and protein–glutathione mixed disulfides, were determined by the procedure of Akerboom and Sies (1981). A fixed concentration of GSSG was added to the contaminated samples to quantify the efficiency of the separation. The recovery of GSSG was over 95%. Unless otherwise specified, the fine biochemicals were purchased from Sigma (USA). All the other reagents were of analytical grade and of the highest purity.

Statistical Analysis

The statistical significance of the differences in biochemical parameters was determined by Student's *t*-test. The normality of data and homogeneity of variance was checked previously.

Results

The pollution status of sampling areas was determined by measuring several metals and organic xenobiotics in fish or sediments. Table 1 shows the Cu, Fe, Mn and Zn contents in *Mugil* sp. (grey mullet) livers and the pollution ratios obtained by comparing the animals from the Huelva Estuary (contaminated) with those from Cádiz Bay (reference). Fish from both locations showed very similar Mn and Zn contents but animals from the Huelva Estuary accumulated higher concentrations of Cu, Fe and total metals than fish from Cádiz Bay. Table 2 summarises the contents of several polychlorinated biphenyls, polyaromatic hydrocarbons and pesticides in sediments from the reference and contaminated sites. Polluted sediments contained 1.1–4.6-fold higher amounts of several polyaromatic hydrocarbons, with a 1.4-fold increase in the total PAH level. Polychlorinated biphenyls also increased in the contaminated sediments (1.8–5-fold), with a total PCB increase of 2.6-fold, particularly those with high chlorine content. No hexachlorobenzene was detected in the sediments and the amount of lindane was similar in both samples. Comparatively, the amount of DDT and related metabolites was much higher (7.8-fold) in the Huelva Estuary.

Because production of reactive oxygen species is catalysed by Cu, Fe and some organic compounds (Sies 1986), the high contents of redox-active metals and organic xenobiotics in the Huelva Estuary prompted us to investigate whether fish from this polluted area were under oxidative stress. Table 3 summarises the activities of several antioxidant enzymes, which were analyzed in either cell-free extracts or microsomes, and those of two of the main detoxifying enzymes. All the enzymatic activities assayed were enhanced in fish from the contaminated site. The antioxidant enzymes included three primary defenses against oxidative stress, superoxide dismutase, catalase and glutathione peroxidase, as well as two ancillary enzymes, glucose-6-phosphate dehydrogenase and glutathione reductase.

TABLE 2. Organic xenobiotic levels in sediments from areas with different pollution levels[a].

Compounds	Reference (R)	Contaminated (C)	Ratio C:R	VC(%)
Polyaromatic hydrocarbons				
Fluoranthene	15.0	15.8	1.1	21
Benzo(*b*)fluoranthene	7.9	11.5	1.5	17
Benzo(*k*)fluoranthene	3.0	4.4	1.5	43
Benzo(*a*)pyrene	5.7	7.9	1.4	27
Benzo(*g,h,i*)perylene	1.0	4.6	4.6	23
Total PAH's	32.6	44.2	1.4	
Polychlorinated biphenyls				
2,4,4′-Trichlorobiphenyl	<0.1	<0.1		
2,2′,5,5′-Tetrachlorobiphenyl	<0.1	<0.1		
2,2′,4,5,5′-Pentachlorobiphenyl	<0.1	0.5	>5.0	13
2,2′,3,4,4′,5′-Hexachlorobiphenyl	0.5	0.9	1.8	13
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	0.4	0.9	2.3	9
Total PCB's	0.9	2.3	2.6	
Pesticides				
Hexachlorobenzene	<0.1	<0.1		
ΣDDTs	0.9	7.0	7.8	13
1,2,3,4,5,6-γ-hexachlorocyclohexane	0.2	0.1	0.5	47
Total pesticides	1.1	7.1	6.5	

[a] Organic xenobiotics are expressed as ppb of dry weight. Variation coefficients [VC = $(\sigma_n/x) \cdot 100$] are expressed in percentages.

TABLE 3. Antioxidative and detoxifying enzymatic activities determined in livers of grey mullet (*Mugil* sp.) living in areas with different pollution levels[a].

Activities	Reference (R)	Contaminated (C)	Ratio C:R
Total glutathione peroxidase[b]	26.5 ± 1.4	54.7 ± 0.4***	2.06
Se-dependent glutathione peroxidase[b]	7.6 ± 1.1	23.6 ± 3.2***	3.11
Superoxide dismutase[c]	8.3 ± 1.0	11.8 ± 0.4**	1.42
Catalase[c]	278.9 ± 12.7	409.7 ± 16.9***	1.47
Glucose-6-phosphate dehydrogenase[b]	70.9 ± 2.6	112.6 ± 2.7***	1.59
Glutathione reductase[b]	19.7 ± 4.1	25.0 ± 3.8	1.27
Soluble glutathione transferase[c]	0.50 ± 0.01	0.77 ± 0.03***	1.55
Microsomal glutathione transferase[b]	14.6 ± 5.5	162.6 ± 12.5***	11.14
Etoxyresorufine-*O*-deethylase[d]	<0.01	15.06 ± 3.10***	>1506

[a] Cell-free extracts and microsomal fractions from livers of fishes from a clean reference site or a contaminated area were analyzed as described in "Materials and Methods." Values are means ± SD of three independent determinations (**$P < 0.01$; ***$P < 0.001$).

[b] mU/mg protein.

[c] U/mg protein.

[d] pmol · min^{-1} · mg protein^{-1}.

TABLE 4. Redox status of glutathione and lipid peroxidation in livers of grey mullet (*Mugil* sp.) living in areas with different pollution levels.

Parameter	Reference (R)	Contaminated (C)	Ratio C:R
Total glutathione[a]	1090 ± 367	1140 ± 175	1.05
GSSG[a]	50 ± 5	80 ± 9***	1.60
% of total glutathione	4.59	7.01	1.53
ProteinS-SG[a]	50 ± 18	90 ± 10***	1.80
% of total glutathione	4.59	7.89	1.72
Ratio GSSG/GSH	0.096	0.160	1.67
Malondialdehyde[b]	0.886	0.256	0.29
Hidroperoxides[c]	0.420	0.254	0.60

[a] Nanomoles of GSH equivalent per gram of fresh weight, mean values ± SD of three independent determinations (***$P < 0.001$).

[b] Absorbance at 535 nm after substraction of the non-specific absorbance at 520 nm.

[c] μM.

Glutathione peroxidase showed the highest increase, 2.1-fold for total activity and 3.1-fold for Se-dependent activity. Increases in the other antioxidant and ancillary enzymes ranged from 1.6-fold for glucose-6-phosphate dehydrogenase to 1.3-fold for glutathione reductase. The increased activity of these antioxidant enzymatic defences suggested that animals from the Huelva estuary were exposed to oxidative stress. The detoxifying status of grey mullets was monitored by assaying glutathione transferase from both cytosol and microsomes, and the ethoxyresorufine-*O*-deethylase activity associated with cytochrome P-450 1A1 (Goksøyr and Förlin 1992). As shown in Table 3, the activities of these two detoxifying enzymes were significantly higher in fish from the Huelva Estuary. Soluble glutathione-S-transferase activity increased 1.6-fold and that of the microsome-associated isoenzyme 11-fold in contaminated fish. No EROD activity was detected in liver microsomes from reference fish but animals from the Huelva Estuary showed an activity of 15.06 pmols · min^{-1} · mg^{-1} of protein. Thus, this phase I detoxifying enzyme gave the greatest difference (>1506-fold).

The redox status of glutathione was analyzed (Table 4) to evaluate further the oxidative stress suggested by the increased activities of antioxidant enzymes in contaminated fish. No significant difference was found in total glutathione content although the glutathione status of contaminated fish shifted clearly towards a more oxidized form. This was clearly shown by the 1.6-fold and 1.8-fold increases in the levels of GSSG and protein–glutathione mixed disulfides, respectively. In short, the ratio of oxidized to reduced glutathione increased 1.7-fold in contaminated animals, thus confirming that they were subjected to oxidative stress. The extent of lipid peroxidation should also be modified by oxidative stress. Lipid peroxidation can be analyzed directly by measuring the levels of lipohydroperoxides (Peinado et al. 1986) or indirectly by determining the levels of malondialdehyde, a product of lipohydroperoxide decomposition (Gutteridge 1984). Table 4 shows that fish from the Huelva Estuary had low levels of lipid peroxidation. The malondialdehyde content of contaminated fish was only 0.3-fold that of reference fish, in agreement with the decrease in the lipohydroperoxide level (0.6-fold).

Discussion

The metal contents determined in fish from the Huelva Estuary agree with previous reports showing that Cu and Fe accumulate in animals and sediments from this site, while their concentrations decrease with distance from the site (Establier 1972; Rodríguez-Ariza et al. 1992). Contamination of the Huelva Estuary was not restricted to metals, but also included different kinds of organic xenobiotics, such as PAHs, PCBs, and organochlorine pesticides, as reflected in the increased glutathione-S-transferase activities previously described in molluscs (Rodríguez-Ariza et al. 1992). Oxidative stress is induced by metal-catalyzed processes and by metabolism of some organic xenobiotics (Sies 1986; Winston 1991). In bacteria, reactive oxygen species induce the coordinated synthesis of antioxidant enzymatic activities, such as catalase, alkylhydroperoxide reductase, Mn-superoxide dismutase, glucose-6-phosphate dehydrogenase, and exonuclease IV (Farr and Kogoma 1991; Demple 1991). No such coordinated genetic control of antioxidant defenses has been described in eukaryotes. Nevertheless, a H_2O_2-resistant hamster cell line shows significantly higher catalase, superoxide dismutase, glutathione transferase, and Se-dependent glutathione peroxidase activities, and enhanced levels of GSH (Spitz et al. 1990a, b). The present report shows that several antioxidant enzymes increased simultaneously in contaminated fishes, in agreement with our previous results in molluscs (Rodríguez-Ariza et al. 1992).

The biochemical analyses of fish from two areas with different levels of metal and organic contamination indicate that animals from the polluted area had been exposed to oxidative stress. Up to 15% of intracellular glutathione is recovered in rat hepatocytes as protein mixed disulfides during oxidative stress (Bellomo et al. 1987). Here we demonstrate that contaminated fish had an oxidized glutathione redox status, with high levels of both GSSG and protein-glutathione mixed disulfides while total glutathione content remained unchanged. Nearly 15% of the total glutathione was in the oxidized form in contaminated animals, compared to 9% for reference fish. While this last data could indicate a somewhat stressed condition for the reference fish, it should be noticed that total hepatic glutathione was determined, and not intracellular glutathione which would be less oxidized because GSSG produced under oxidative stress is excreted to extracellular fluids (Sies, 1986). The more oxidized glutathione redox status of contaminated fish does not contradict their higher glutathione reductase activity, as GSSG reduction requires sufficient NADPH concentration in addition to an active glutathione reductase. Oxidative stress has been reported to lower NADPH concentration (Brigelius 1985) which, along with the increased glutathione peroxidase activity, would explain the oxidized glutathione status.

Antioxidative activities increased in fish from the Huelva Estuary, acting as a possible protection mechanism against chronic oxidizing conditions. This adaptive response included enzymes that decompose reactive oxygen species (superoxide dismutase and catalase), attack peroxidized biomolecules (glutathione peroxidase) or recycle low MW antioxidants into their reduced forms (glucose-6-P dehydrogenase and glutathione reductase). Among soluble enzymes, the highest increase was detected in glutathione peroxidase, particularly the Se-dependent isoenzyme, according to the predominant role of this isoenzyme in protection against lipid peroxidation in mammals and fish (Burk and Lawrence 1978; Steadman et al. 1991). Fish are better protected than mammals against lipid peroxidation, due to their increased glutathione peroxidase activity and their high levels of vitamin E (Winston and Di Giulio 1991). It should be noticed that microsomal glutathione transferase increased 11-fold. In mammals this enzyme displays, in addition to its conjugating activity, Se-independent glutathione peroxidase activity, suggesting a protective role against lipid peroxidation of microsomal and plasma membranes (Mosialou and Morgenstern 1989).

The low level of lipid peroxidation in contaminated fish, indicated by the simultaneous decreases in both MDA and lipohydroperoxide contents, is in agreement with high glutathione peroxidase and microsomal glutathione transferase activities as well as with increased GSSG and protein S-SG contents, derived from glutathione peroxidation. We have previously reported that polluted molluscs from the Huelva Estuary display high glutathione peroxidase activities in parallel with low MDA levels. In fact, *Crassostrea gigas*, the species with the highest increase in glutathione peroxidase, showed the lowest MDA content (Rodríguez-Ariza et al. 1992). Our data on fish and molluscs from the Huelva Estuary do not agree with the increased MDA levels found by others after short-term exposure of fish to metals and organic xenobiotics (Radi and Matkovics 1988; Wofford and Thomas 1988; Winston and Di Giulio 1991). Nevertheless, it should be noticed that animals living in contaminated environments must adapt to adverse circumstances. Thus, fish and molluscs from the Huelva Estuary have probably adapted to the chronic oxidizing conditions in which they live; their high degree of glutathione peroxidase activity and that of other antioxidant enzymes explain the low level of lipid peroxidation while their oxidized glutathione status confirms that they have been exposed to oxidative stress.

Previous reports concerning the response of fish antioxidant defences to metal and organic xenobiotic exposure have shown variable results. Acute exposure of carp to Cu^{2+} resulted in increased glutathione peroxidase activity and high MDA levels in most tissues while superoxide dismutase and catalase activities decreased. In comparison, Zn^{2+} yielded variable responses although liver glutathione peroxidase and superoxide dismutase increased at a high dose (Radi and Matkovics 1988). Increases in hepatic GSH concentrations and lipid peroxidation were found in fishes exposed to Cd and organic xenobiotics (Thomas and Wofford 1984; Wofford and Thomas 1988). Fishes exposed to polyaromatic hydrocarbons showed increased superoxide dismutase, catalase and aryl-hydrocarbon hydroxylase activities as well as high GSH, GSSG, and MDA levels (Winston and Di Giulio 1991). Significant increases in aryl-hydrocarbon hydroxylase, glucose-6-P dehydrogenase, 6-phosphogluconate dehydrogenase and cytochrome P-450 were observed in fish living in polluted seawater although decreased glutathione peroxidase and glutathione transferase activities were also detected (Bagnasco et al. 1991).

The increased levels of organic xenobiotics (PCBs, PAHs, pesticides) detected in sediments in the Huelva Estuary clearly explain the high increase in EROD activity (>1500-fold), in agreement with the well established role of fish cytochrome P-450 induction as a bioindicator of organic pollution (Stegeman and Lech 1991; Goksøyr and Förlin 1992). In contrast to EROD activity, the usefulness of phase II enzyme glutathione transferase in monitoring aquatic pollution has not been well established. Significant increases in this enzyme were observed in trout injected with β-naphthoflavone (Goksøyr et al. 1987) and Aroclor-injected catfish (Ankley and Blazer 1988), although no differences could be detected in fishes injected with 3-methylcholantrene (James et al. 1988). Our results suggest that glutathione transferase activity can also be a useful tool for monitoring environmental pollution, as previously shown for molluscs (Rodríguez-Ariza et al. 1992). In mammals, the expression of cytochrome P-450 genes is coordinated with that of four phase II enzymes, including glutathione transferase, through the interaction of the Ah receptor with xenobiotics (Nebert et al. 1990). A protein with the properties of the Ah receptor has been recently isolated and characterized in a trout hepatoma cell line (Lorenzen and Okey 1990), thus implying that fish may have a regulatory mechanism similar to that in mammals. Rat Y_a-glutathione transferase is also induced by oxidative stress (Rushmore et al. 1991).

There is a growing concern with respect to the role of oxidative damage in the harm induced by different types of environmental pollutants. In fact, oxidative lesions have been recently related to liver tumour formation in fish from polluted environments (Malins and Haimanot 1991). Our results indicate that fish fight back against oxidative stresses derived from environmental pollution through the coordinated increase in the activities of a battery of antioxidant and detoxifying enzymes. Such an adaptive response could be useful for the biomonitoring of environmental pollution, as has been recently proposed (Livingstone 1991; Winston and Di Giulio 1991; Rodríguez-Ariza et al. 1992).

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Accumulation of Persistent Pollutants in Normal and Dwarfed Arctic Char (*Salvelinus alpinus* sp. complex)

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Hammar, J., P. Larsson, and M. Klavins. 1993. Accumulation of persistent pollutants in normal and dwarfed Arctic char (*Salvelinus alpinus* sp. complex). Can. J. Fish. Aquat. Sci. 50: 2574–2580.

Two extreme growth fractions of introgressed sympatric populations of the Arctic char (*Salvelinus alpinus*) species complex from Lake Blåsjön, northern Sweden, were sampled for individual concentrations of persistent pollutants originating from atmospheric deposition. Slow growing char (dwarfs) had significantly higher levels of ΣPCB (sum of PCB congeners found) and *p,p*-DDE (dominating DDT-compound) than fast growing char (normals). Besides demonstrating a highly divergent growth pattern the introgressed populations also inhabit a great range of depths, and the seasonal food consumption rates may differ between extreme growth fractions, although the same major invertebrates (introduced *Mysis relicta* and *Pallasea quadrispinosa*) are consumed over the year. However, of the life history parameters differing in late spring, individual growth rate was the most important factor explaining the variation in the levels of the pollutants studied. The results support the hypothesis of a biomass "dilution" of pollutants in fish, illustrated by lower levels in fast growing char and higher levels in the slow-growing dwarfs. Individuals with alternative growth strategies in sympatric conspecific communities thus provide unique opportunities to study growth rate as a variable influencing levels of persistent pollutants in fish.

Dans le lac Blasjon, dans le nord de la Suède, il existe deux groupes à la croissance radicalement différente au sein des populations sympatriques introgressées du complexe d'espèces de l'omble chevalier (*Salvelinus alpinus*). Ces groupes ont été échantillonnés en vue de la mesure de la concentration de certains contaminants persistants d'origine atmosphérique. Chez les ombles à la croissance lente (nains), la concentration de ΣBPC (la somme des congénères de BPC trouvés) et du *p,p*-DDE (fraction dominante du DDT) étaient trouvés en concentrations significativement supérieures à ce qu'elles sont dans les tissus des ombles à croissance normale (normaux). En plus d'une courbe de croissance fortement divergente, les populations introgressées se tiennent à l'intérieur d'une vaste plage de profondeurs; en outre, les taux saisonniers d'alimentation peuvent différer entre les deux groupes même si ce sont les mêmes principaux invertébrés qui sont consommés au cours de l'année (*Mysis relicta*, introduite, et *Pallasea quadrispinosa*). Il demeure toutefois que, des paramètres du cycle biologique qui diffèrent à la fin du printemps, le taux de croissance individuel est le principal facteur pour expliquer l'écart de concentration des contaminants étudiés. Les résultats viennent étayer l'hypothèse à l'effet qu'il se produit une « dilution » des contaminants dans la biomasse des poissons, du fait que la concentration est inférieure chez l'omble à la croissance rapide et supérieure chez l'omble nain. Au sein de communautés de congénères sympatriques, ceux qui emploient des stratégies différentes de croissance nous offrent une occasion unique d'étudier le taux de croissance à titre de variable qui influence la concentration de contaminants persistants dans les tissus du poisson.

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Animal populations inhabiting the higher latitudes and other remote areas, far from human concentrations, suffer an increasing risk of being exposed to various persistent pollutants originating from atmospheric deposition. Several investigations have demonstrated the significance of such transport for the northern boreal and Arctic regions (e.g., Bowes and Jonkel 1975; McNeely and Gummer 1984; Oehme 1991; see also Hammar 1989), where compounds such as hexachlorocyclohexanes, DDT and metabolites, and polychlorinated biphenyls (PCBs) have been traced in landlocked fish (e.g., Risebrough and Berger 1971; Johnson et al. 1988; Swackhamer and Hites 1988; Muir et al. 1990; Rasmussen et al. 1990; see also Wania and Mackay 1993). High levels have especially been recorded in long-lived predators such as northern pike (*Esox lucius*), burbot (*Lota lota*), and lake trout (*Salvelinus namaycush*).

Within the ecosystem, persistent pollutants reach predatory fish mainly through their prey (Jensen et al. 1982; Oliver and

Niimi 1989; Rasmussen et al. 1990; Madenjian et al. 1993). Consequently, in addition to abiotic factors constituting the pollutant's chemical characteristics (lipophilicity, adsorption behavior and persistence) and its bioavailability (speciation to particles and humic compounds) (for references see Johnson et al. 1987), the levels in fish are also affected by physiological factors, such as assimilation efficiency of the gut, and the body growth rate (Connolly 1991; Larsson et al. 1991).

Several mathematical models have recognized the growth rate of the individual fish as an important variable in the accumulation of persistent pollutants (Jensen et al. 1982; Thomann and Connolly 1984; Connolly 1991; Borgmann and Whittle 1992). In general a fast growing fish attains lower concentrations of persistent pollutants than a slow growing one, due to "dilution" of the pollutants by the tissue. For the individual fish this means that the levels of persistent pollutants increase slowly when growth rate is rapid (such as in early life stages), and when the growth rate declines (proceeds towards an asymptote as in later life stages) the levels of pollutants increase faster (Jensen et al. 1982). However, these hypotheses/conclusions are difficult to verify on fish in the field, since changes in the choice of food and habitat in general shift with age and size of the fish, thereby altering the uptake of pollutants.

Northern lakes inhabited by single or few fish species with comparably wide niches may provide unique opportunities to test growth rate as a variable affecting individual pollutant levels in fish. True sympatric populations of Arctic char demonstrate as a rule alternative life history strategies (e.g., Hammar 1989). These express among a series of traits major differences in growth; i.e., the populations are commonly referred to as normal and dwarfed char, respectively (e.g., Nyman 1972, 1984). Besides growing differently, sympatric char populations may also segregate in terms of diet and thus their parasite fauna, choice of habitat, age and size at maturity, date and site of spawning, and life span (e.g., Nilsson and Filipsson 1971; Klemetsen and Grotnes 1975, 1980; Henricson and Nyman 1976; Hammar 1984; Hammar and Filipsson 1988).

However, in exploited northern reservoirs where *Mysis relicta* have been introduced in order to compensate for the loss of natural littoral fish food organisms (Fürst et al. 1984), the mechanisms for segregation between sympatric Arctic char have been more or less eliminated (Nyman 1972; Fürst et al. 1978; Hammar 1984). Consequently under such special "monoculture" circumstances, highly introgressed char populations diverging extensively in growth, can be tested for levels of persistent pollutants, evaluating the significance of individual variation in growth. The general exposure of persistent pollutants for such populations would be identical, i.e., in respect of the atmospheric deposition on the lake. The fact that *Mysis relicta* has been recognized as an efficient vector of various pollutants from sediment surface to the fish community (Evans et al. 1982; Evans and Lasenby 1983; van Duyn-Henderson and Lasenby 1986; Rasmussen et al. 1990; Hammar et al. 1991a, b; Klump et al. 1991) further promotes the preference of such a system.

To test the hypothesis that slow growing individuals contain higher levels of persistent pollutants than faster growing ones, two extreme growth fractions of the introgressed Arctic char collected from Lake Blåsjön, northern Sweden, have been analyzed. *Mysis relicta* was previously introduced into Lake Blåsjön and is today the predominant food resource for the salmonid populations present (Fürst et al. 1978). There is no historic evidence of sources of pollution other than from the atmosphere.

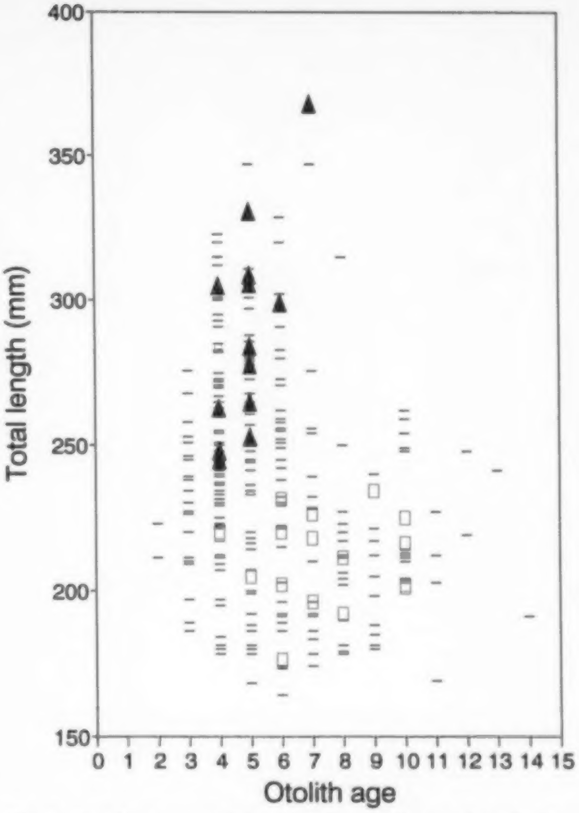

FIG. 1. Growth shown as total length by otolith age of Arctic char ($n = 277$) caught with gillnets and hook in the husbandry fishery of Lake Blåsjön during a radio-cesium monitoring program in 1986–1990. Slow-growing char shown with empty squares, and fast-growing char shown with filled triangles were caught in May 1990, and analyzed for persistent pollutants in the present study.

Material and Methods

Study Area

Lake Blåsjön (64°48′N, 14°10′E), with an area of 43 km^2 and a maximum depth of 145 m, forms the first upper reservoir in River Faxälven. The lake is located at 438 m a.s.l. and is, except for the small village Stora Blåsjön and a narrow belt of coniferous wood, surrounded by a barren alpine watershed. The water level is regulated 13.3 m for hydroelectric purposes, and in order to compensate for the loss of littoral invertebrates due to the regulation, the opossum shrimp, *Mysis relicta*, and the amphipod *Pallasea quadrispinosa* were introduced in 1964. The fish fauna comprise two highly introgressed taxa within the Arctic char (*Salvelinus alpinus*) species complex, brown trout (*Salmo trutta*) and the recently introduced European minnow (*Phoxinus phoxinus*). The reservoir and its sympatric Arctic chars have been extensively studied since the 1940's (e.g., Määr 1949; Nilsson 1961; Nyman 1972; Fürst et al. 1978; Hammar et al. 1983, 1991a; Hammar 1984).

Sampling

Two extreme growth fractions of Arctic char were collected with benthic gillnets in Lake Blåsjön, May 22–29 1990 (Fig. 1). Fast growing char were mostly caught in shallow water whereas slow growing char were mainly caught at deeper bottoms. The fish were put in a deep freezer shortly after capture, and transported frozen to the laboratory. For each fish the total length and full weight was measured (to the nearest mm and 0.1 g, respectively). Sex, sexual maturity (1–7) and muscle color (1–9) of flesh were also recorded (see Hammar and Filipsson 1985), and the age was determined using otoliths, according to criteria given by Nordeng (1961). The relative volume of the various food items in their stomachs were estimated. For various group comparisons the individuals were separated as "normals" (n = 14) or "dwarfs" (n = 16) by characteristics given by Hammar (1984); e.g. general appearance, size to age relationship, size and age at maturity, flesh coloration and the depth of capture.

Analyses

From each fish, a segment of 5–15 g was dissected from the lateral muscle. The segment was homogenized, and extracted by acetone/hexane according to the method described by Larsson et al. (1991). The hexane fraction was evaporated in a water bath (70°C) to dryness. The remainder was redissolved in acetone and again evaporated to complete dryness to remove all water. The remains which were regarded as fat were then weighed to the nearest 0.01 mg. The fat residue, containing persistent pollutants, was redissolved in 3 mL hexane and treated three times with concentrated H_2SO_4 to oxidize and remove the fat. The cleaned-up extract was evaporated to 200 μL, treated with silica-gel (with 33% concentrated H_2SO_4 added) and finally analyzed for persistent pollutants by capillary gas-chromatography/ECD.

PCBs and p,p-DDE were analyzed by a Shimadzu GC/ECD equipped with a 30 m DB 5 capillary column. H_2 (1.5 mL/min) and N_2 (50 mL/min) were used as carriers and make-up gas, respectively. Injection was split/splitless run in the splitless mode, injection temperature was 310°C, and a program was performed of an initial temperature 80°C for 15 min, 4°C/min to a final temperature of 300°C which was kept for 15 min.

PCB congeners (ΣPCB, 41 congeners) were identified and quantified according to Mullin et al. (1984) and Schulz et al. (1989), with octachloronaphthalene as retention- and response standard, together with regular analysis of Aroclor 1254/1260. As internal standards PCB 53 (2,2′,5,6′-tetrachlorobiphenyl) and pentachlorobenzene were used. Spiked fish extracts showed recovery of 77% (70–84%, n = 8).

Statistics

The non-parametric Mann–Whitney U-test, was used for statistical comparisons of individuals in groups of normal and dwarfed char. Correlations between individual concentrations of pollutants and various individual parameters and their covariates were identified in a matrix of linear regressions of log-transformed variables. The influence of the adjusted coefficients of determination for the various independent variables were tested in a stepwise multiple regression analysis. The effect of growth, phenotype, state of sexual maturity, sex and age were tested on the response of the concentrations of ΣPCB and p,p-DDE in an analysis of variance (ANOVA). The statistical software used was SPSS/PC+ 4.0.

Results

Total length and full weight of the fraction of normally growing char were always higher than for dwarfs (Table 1, Two-tailed Mann-Whitney U-test, $p < 0.001$). Also Fulton's condition factor (Ricker 1975) ($p = 0.003$) and a coarse growth index (full weight/age) ($p < 0.001$) revealed higher values in normally growing char. The sex distribution was skewed ($\chi^2 = 5.14$, $p < 0.05$) with a dominance of females (9/5 = 1.8) among normal char and dominance of males among dwarfs (4/12 = 0.3). Whereas all dwarfs were sexually mature or spent, only four normal char were mature or had spawned previously. The dwarfs were older than the normal char (Mann-Whitney U-test, two-tailed, $p < 0.001$); the otolith age ranged between 4 and 7 years in normal char (mean ± std: 4.9 ± 0.8) and between 4 and 10 yr in the dwarfs (mean ± std: 7.4 ± 1.9). The tissues of the normal char were more red than in dwarfed char ($p < 0.001$).

Both groups of char had been actively feeding at the end of May, and in both samples the stomach contents were dominated (ca. 65%) by *Mysis relicta* (Table 2). There were only minor differences in the degree of filled stomachs or the volume of the various ingredients. Among the normal char the additional components were dominated by *Pallasea quadrispinosa*, and in the dwarfs by *Pallasea quadrispinosa* and midge larvae and pupae together.

The fat content was higher in muscular tissue of normal char (mean 1.3 ± 0.3 %) than in dwarfs (mean 1.1 ± 0.2%) (Mann-Whitney U-test, $p = 0.015$). Dwarfs had higher levels of ΣPCB (sum of all PCB congeners) or p,p-DDE than normal char (Fig. 2, Mann-Whitney U-test, $p < 0.001$) both on fresh-weight and extractable fat weight basis. Geometrical mean ΣPCB-level for dwarfs was 1295 ng/g extractable fat (13.6 ng/g fresh weight) and for the normal form 302.5 ng/g extractable fat (3.98 ng/g fresh weight). For p,p-DDE (the dominating DDT compound) the geometrical mean was 360.5 ng/g extractable fat (3.80 ng/g fresh weight) for dwarfs and 89.9 ng/g extractable fat (1.18 ng/g fresh weight) for normal growing fish.

There were, however, no differences between dwarfs and normal char in their total load of ΣPCB ($p > 0.31$) or p,p-DDE ($p > 0.44$) (ng ΣPCB or p,p-DDE/fish, Mann-Whitney U-test). No significant differences between sexes of either growth fraction was recorded for ΣPCB or p,p-DDE (Mann-Whitney U-tests).

In a matrix of correlation analyses of all available data in logarithmic form of the two pooled samples, growth index, full weight, total length and otolith age were highly correlated with the concentrations of ΣPCB and p,p-DDE, respectively, the correlations with p,p-DDE in general being higher than those with ΣPCB (Table 3).

The concentrations of PCB and DDE thus increase with decreasing growth (Fig. 3), full weight, total length and Fulton's condition index, and with increasing age. The initial maturity classification, which is not a linear physiological gradient, gave for both ΣPCB and p,p-DDE the highest single positive correlation. However, pooling immature with spent char, and mature individuals with those spent fish which were going to spawn again the succeeding fall, which are physiologically more comparable, weakened these strong correlations. Char which were going to spawn during upcoming fall thus had slightly higher levels of ΣPCB and p,p-DDE than immature or "resting" char. Furthermore the variable growth (weight/age) gave higher correlation coefficients than age, and full weight gave higher

TABLE 1. Individual descriptions of the two forms of Arctic char, caught in Lake Blåsjön, in May 1990, and their concentrations of ΣPCB and *p,p*-DDE (ng/g fresh weight). Concentrations of pollutants in char on extractable fat weight basis are given in Fig. 2.

FORM D/N	LENGTH mm	WEIGHT g	GROWTH g/yr	SEX ♂/♀	MAT 1–6[a]	AGE y	COL 1–9[b]	FAT %	PCB ng/g	DDE ng/g
D	216	77.9	7.8	♂	6	10	1	0.88	10.0	2.8
D	192	50.9	6.4	♂	6	8	1	1.13	33.2	9.1
D	211	82.2	10.3	♂	6	8	1	0.81	27.1	7.5
D	218	89.4	12.8	♀	5	7	1	1.24	28.9	7.9
D	226	103.7	14.8	♂	5	7	1	0.92	7.5	2.2
D	225	82.5	8.3	♂	5	10	1	0.73	29.3	7.3
D	234	93.2	10.4	♂	5	9	1	1.05	5.5	1.6
D	202	71.6	7.2	♂	6	10	1	1.59	3.9	1.2
D	220	98.8	24.7	♂	5	4	6	1.18	9.8	2.9
D	220	97.5	16.3	♂	5	6	6	1.21	5.3	1.6
D	196	74.5	10.6	♀	6	7	3	1.10	21.8	5.8
D	231	110.9	18.5	♀	5	6	1	0.90	19.3	5.3
D	202	72.1	12.0	♀	5	6	3	1.38	22.5	5.8
D	205	73.1	14.6	♂	3	5	1	0.90	5.5	1.6
D	201	63.8	6.4	♂	6	10	1	0.90	18.3	5.1
D	176	53.2	8.9	♂	6	6	1	1.31	23.8	6.0
N	331	331.4	66.3	♀	2	5	6	1.01	0.9	0.3
N	305	247.4	61.9	♂	2	4	6	1.14	4.4	1.3
N	309	277.7	55.5	♀	2	5	7	1.39	4.8	1.4
N	284	222.9	44.6	♂	2	5	5	1.19	1.6	0.5
N	263	169.2	42.3	♂	2	4	5	1.59	8.4	2.4
N	368	491.7	70.2	♀	5	7	7	1.39	7.7	2.1
N	306	273.1	54.6	♀	2	5	7	2.07	2.8	0.8
N	278	202.8	40.6	♀	3	5	6	1.26	7.7	2.2
N	245	143.7	35.9	♀	2	4	5	1.56	5.2	1.5
N	265	169.1	33.8	♂	2	5	5	1.71	8.9	2.6
N	253	147.9	29.6	♂	2	5	5	1.10	4.3	1.3
N	248	146.9	36.7	♀	2	4	5	1.32	2.2	0.7
N	306	257.6	51.5	♀	3	5	5	1.30	3.4	1.0
N	299	258.7	43.1	♀	3	6	7	0.83	3.9	1.2

[a] Sexual maturity: 2 = maturing, 3 = mature, 5 = spent fall 1989, 6 = spent fall 1989, mature 1990.
[b] Tissue coloration: 1 = white, 3 = light pink, 6 = pink, 9 = red, from Hammar and Filipsson 1985.

correlations than total length and Fulton's condition index. The fat content was correlated with various size and condition traits but not with the concentration of pollutants.

In multiple stepwise regression analyses with growth, full weight, and the fat content as three independent variables of significance, the concentrations of ΣPCB and *p,p*-DDE in the Arctic char were explained to 45.6 and 46.4%, respectively, by the growth index solely ($p < 0.001$). If growth is added as a covariate in analyses of variation (ANOVA), no significant differences between the levels of ΣPCB and *p,p*-DDE were recorded when normals or dwarfs phenotypes ($p = 0.444, 0.435$), state of sexual maturity ($p = 0.764, 0.769$), sex ($p = 0.221, 0.238$) or age ($p = 0.184, 0.154$) were used to distinguish groups. When using age or the sexual maturity stage as a covariate, the differences in concentrations of pollutants were still significant between the two phenotypes. This confirmed that growth was the major variable explaining the differences in concentrations of ΣPCB and *p,p*-DDE.

Dominating PCB congeners (numbered according to IUPAC, Ballschmiter and Zell 1980) in the Arctic char from Lake Blåsjön were PCB 149 (co-eluting compounds PCB 118+123), PCB 153 (co-eluting compounds PCB 105+132), PCB 138 (co-eluting compounds PCB 158+160), PCB 187 and PCB 180.

TABLE 2. Stomach content of two sympatric growth fractions of Arctic char, Lake Blåsjön, May 1990. The figures represent relative percentage of volume.

Food organisms	Normal char	Dwarfed char	Total
Mysis relicta	64.6	60.9	63.2
Pallasea quadrispinosa	30.9	22.7	27.9
Pisidium sp.	1.7	2.0	1.8
Ephemeroptera larvae	0.4	0.0	0.3
Plecoptera larvae	0.2	0.0	0.1
Chironomidae larvae	0.7	10.0	4.1
Chironomidae pupae	1.5	4.4	2.6
Degree of filling	4.1	1.9	2.9
n	15	18	33

Discussion

As predicted by theoretical models (Jensen et al. 1982; Connolly 1991), the growth rate of individual Arctic char in Lake Blåsjön was confirmed to have a considerable effect on the concentration of persistent pollutants. This was the case both for calculations made on fresh weight basis and on extractable lipid basis, thus showing that the difference in lipid content between

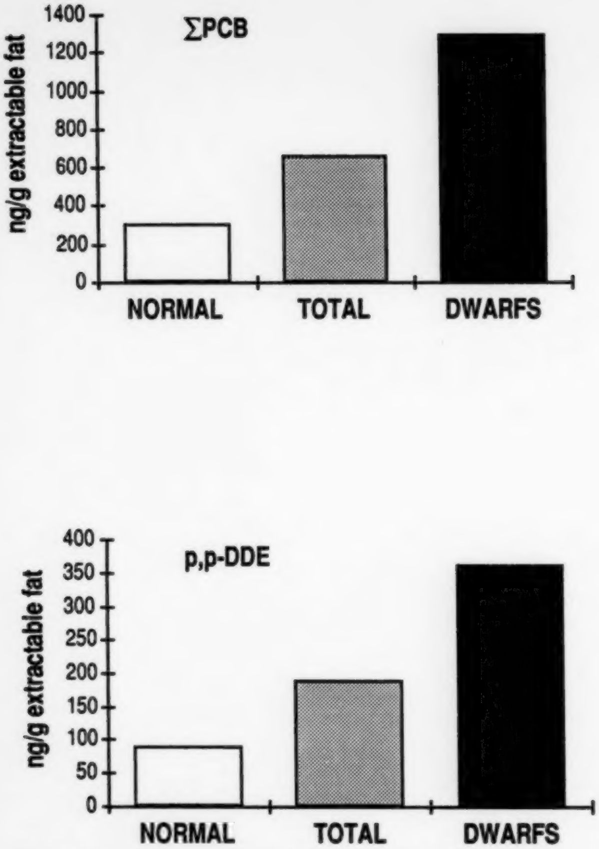

FIG. 2. Concentrations (geometrical means) of persistent pollutants shown as nanograms per gram extractable fat in normal and dwarf forms of Arctic char, caught in Lake Blåsjön, northern Sweden.

the two char groups did not influence the results. This demonstrates that slow-growing forms or slow-growing individuals of a fish population are exposed to higher concentrations of persistent pollutants than forms or individuals with faster growth. Similar conclusions were drawn after the comprehensive analyses of the accumulation of radiocesium in Arctic char in Lake Blåsjön and other nearby lakes after the Chernobyl disaster (Hammar et al. 1991a).

Because growth commonly is a competitive trait for fishes, the exposure to higher pollutant levels in slow-growing fish would decrease fitness to an individual already subjected to lowered competitive ability shown by slower growth rate. Additionally, a further effect of increased pollutant exposure is decreased reproductive capacity (e.g., von Westernhagen et al. 1987; Walker et al. 1991), which thus would reduce fitness for slow growing individuals even more. These conclusions may have consequences for the evolution of taxa or populations with alternative life history strategies in contaminated areas.

However, besides growth rate, a series of other life history characteristics differ between dwarfs and normal char in sympatric populations. Those assumed to affect the levels of persistent pollutants include seasonal differences in choice of habitat and food, the feeding rate, age structure, age at maturity, and the date and duration of spawning activity.

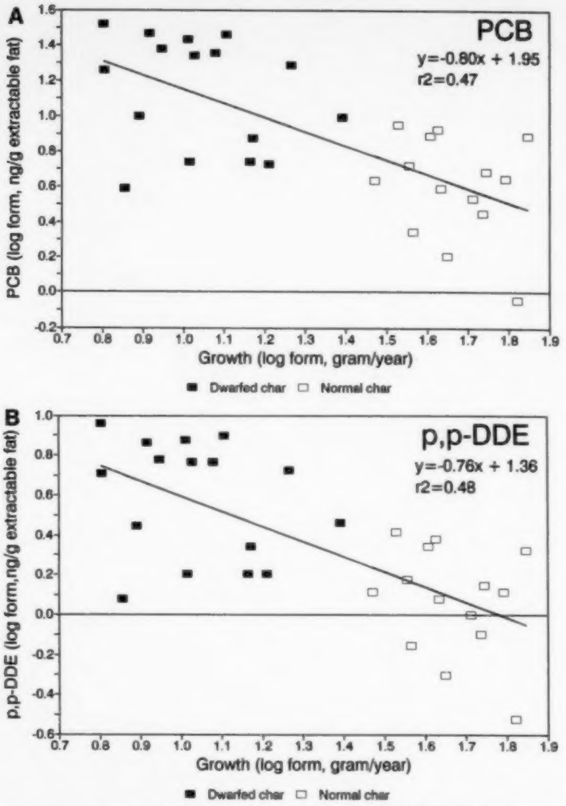

FIG. 3. The relationships between levels of (A) ΣPCB (log form), (B) *p,p*-DDE (log form) and the growth (log form) of Arctic char in Lake Blåsjön, May 1990. The given equations represent the linear regressions.

The stomach contents of the dwarfed and normally growing char collected during the end of May were quite similar. *Mysis relicta* dominates the diet of both char fractions in Lake Blåsjön during most of the colder seasons of the year (Fürst et al. 1978; Hammar 1984; Hammar et al. 1991a). The seasonal variation in food habits, however, demonstrates a major zooplankton dominance in normal char during July–October, whereas the zooplankton peak in the diet of dwarfed char is smaller and occurs during September–October (Hammar 1984). Because *Mysis relicta* acts as a pathway of persistent pollutants from sediment surface to the fish community in North American lakes (Evans et al. 1982; Klump et al. 1991), variation in the annual gross consumption of mysids may amplify the recorded differences. Seasonal diet variations in addition to major differences in choice of habitat and thus temperature, also involve major differences in the feeding rates of Arctic char (Langeland et al. 1991; J. Hammar unpublished data). Thus differences in feeding habits can not be excluded as a factor enhancing differences in pollutant levels between dwarf and normal forms of char found in this study.

With increasing size (and age) Arctic char may eventually switch to a fish diet and may become cannibalistic (e.g., Hammar and Filipsson 1988). This shift in diet may cause a further increase in the exposure to persistent pollutants in piscivorous

TABLE 3. Linear regressions between various traits of Arctic char and their levels of ΣPCB and *p*,*p*-DDE in Lake Blåsjön, May 1990.

Trait	*n*	ΣPCB (log form)		*p*,*p*-DDE (log form)	
		r	*p*	*r*	*p*
Growth (g/yr) (log form)	30	−0.689	<0.001	−0.695	<0.001
Weight (g) (log form)	30	−0.675	<0.001	−0.683	<0.001
Length (mm) (log form)	30	−0.662	<0.001	−0.670	<0.001
Age (yr) (log form)	30	0.485	0.007	0.485	0.007
Condition (log form)	30	−0.393	0.032	−0.397	0.030
Fat Content(%) (log form)	30	−0.274	0.143	−0.283	0.130
Maturity (2–3)	30	0.267	0.153	0.271	0.148

char, especially if the prey consist of dwarfed Arctic char with higher levels of pollutants. The differences in levels of pollutants between two sympatric char populations may then gradually decrease with age.

No significant sexual differences in levels of pollutants were recorded among the char, thus the differences in sex-ratio can not explain differences in levels of pollutants between normal and dwarfed char. Dwarfed Arctic char usually reach sexual maturity at an earlier age than the normal form (e.g., Fürst et al. 1978; Hammar and Filipsson 1988). It has been discussed whether persistent pollutants are significantly eliminated in the gametes of fish (Niimi 1983; Vodicnik and Peterson 1985). If such an elimination process is at work, dwarfed female char may eliminate greater portions of its pollutant burden regularly via eggs, as dwarfed char generally also spawn more frequently and regularly than normal (e.g., Sparholt 1985). Thus, this process should decrease the pollutant burden mainly for female dwarfs, a consequence not recorded in this study where dwarfed females in fact also showed higher levels of pollutants than dwarfed males. Furthermore, individual char which were either spent or were going to spawn the succeeding fall had higher concentrations of pollutants in their muscle tissue, than those individuals which were sexually mature. This result supports the findings that individuals which allocate energy in production of somatic tissue would accumulate less of the pollutants. However, when adjusting for maturity differences, the two different phenotypes still demonstrate significant differences in levels of pollutants. Comparisons between the two forms with respect to their state of sexual maturity is complicated by the fact that the date of spawning differs significantly, and the two samples compared are not in the same sexual phase. Whereas the normal char spawns in early October, the dwarfs start to spawn a month later and ripe dwarfs may still be caught as late as April (J. Hammar 1984, unpublished data).

Although concentrations were lower in normal than dwarf char, there were no significant differences in total amounts of ΣPCB or *p*,*p*-DDE (ng/fish) calculated for fish of the two groups. The total amounts calculated, however, represent a simplification as only muscular tissues were analyzed for persistent pollutants and no other organs. In the calculation we presume that levels are similar within the whole body. On the other hand, levels of pollutants within different organs and tissues in an animal are intercorrelated (Larsson and Lindegren 1987; Lambeck et al. 1991), meaning that high levels in muscular tissue correlate with high levels in other organs, thereby justifying our simplification.

In another Swedish study on Arctic char, Andersson et al. (1988) found the levels of ΣPCB to reach 700–3000 ng/g extractable fat and for ΣDDT (*p*,*p*-DDE + *p*,*p*-DDD + *p*,*p*-DDT) 180–1600 ng/g. Although the char samples of that study represent three different locations, the individual weight of the Arctic char, 78–191 g, and the levels of pollutants were comparable to the char analyzed in Lake Blåsjön. Rasmussen et al. (1990) concluded that levels of PCBs in lake trout (*Salvelinus namaycush*) increased with the trophic level of the food chain. In lakes containing intermediate trophic levels, represented by rainbow smelt (*Osmerus mordax*) and *Mysis relicta*, higher levels of PCBs were recorded in the salmonid predators. Furthermore the levels of PCBs in lake trout increased in lakes located closer to human activity, along a gradient in the Canadian Arctic. For lake trout, levels of PCBs ranged between 700–68 500 ng/g extractable fat. Geometrical means for dwarfs in our study was 1295 ng/g extractable fat and 302.5 ng/g for normal growing fish. Individual lake trout in the study by Rasmussen et al. (1990) were considerably larger (and presumably older, age not given in reference) and thereby piscivorous, explaining their higher levels.

In conclusion, growth rate has been verified as a very important factor for the explanation of differing levels of pollutants in fish. Differences in age structure, sexual maturity cycles and food habits may also contribute to such differences. Populations with alternative growth strategies in sympatric conspecific groups of species, not necessarily restricted to Arctic char, thus provide unique opportunities to further experimental studies on variables influencing levels of persistent pollutants in fish.

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Match/Mismatch between the Abundance of Marine Fish Eggs and Invertebrate Predators: An Analysis of *Calliopius laeviusculus* (Amphipoda: Gammaridae) Population Growth Relative to the Seasonal Spawning Cycle of Capelin (*Mallotus villosus*)[1]

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DeBlois, E.M., and W.C. Leggett. 1993. Match/mismatch between the abundance of marine fish eggs and invertebrate predators: an analysis of *Calliopius laeviusculus* (Amphipoda: Gammaridae) population growth relative to the seasonal spawning cycle of capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 50: 2581–2590.

The amphipod *Calliopius laeviusculus* actively preys on the intertidal eggs of capelin (*Mallotus villosus*). From samples collected in 1988, we found that the patterns of changes in *C. laeviusculus* biomass closely matched changes in capelin egg biomass throughout the capelin egg development period. *Calliopius laeviusculus* and capelin egg biomass exhibited pronounced and overlapping peaks in mid June. Endogenous reproductive patterns influenced by exogenous seasonal stimuli in both *C. laeviusculus* and capelin could facilitate yearly biomass coupling between these two species. *Calliopius laeviusculus* exhibited a semiannual life cycle, overwintering adults releasing young in April, May and July. Cohorts released in April and May reached sexual maturity within the summer, reproduced and produced a second maximum in amphipod biomass at the beginning of August. This second abundance peak appeared to be a numerical response to predation on capelin eggs by the adult *C. laeviusculus* present in mid-summer. Given this response and the reported quiescence of *C. laeviusculus* during the winter, we speculate that amphipod biomass in spring may be strongly linked to feeding conditions in the previous year. The implications of this coupling for capelin egg survival are considered.

L'amphipode *Calliopius laeviusculus* se nourrit activement des oeufs de capelan (*Mallotus villosus*) dans la zone intertidale. À partir d'échantillons prélevés en 1988, nous avons établi que l'évolution de la biomasse de *C. laeviusculus* suit de près celle de la biomasse des oeufs de capelan pendant toute la période de développement de ces oeufs. À la mi-juin, la biomasse de *C. laeviusculus* et celle des oeufs de capelan passent par des pics prononcés et qui se chevauchent. Les phases endogènes du cycle de la reproduction qui sont influencées par des stimulis saisonniers exogènes, tant chez *C. laeviusculus* que chez le capelan, pourraient faciliter le couplage annuel des biomasses de ces deux espèces. On observe chez *C. laeviusculus* un cycle biologique semi-annuel : les adultes qui hivernent libèrent les jeunes en avril, en mai et en juillet. Les cohortes d'avril et de mai atteignent leur maturité sexuelle au cours de l'été, se reproduisent et produisent un deuxième maximum de la biomasse des amphipodes au commencement d'août. Ce second pic d'abondance pourrait constituer une réponse numérique à la prédation sur les oeufs de capelan par les *C. laeviusculus* adultes au milieu de l'été. Compte tenu de cette réponse et de la période hivernale de repos de *C. laeviusculus* dont il est fait état, nous avançons l'hypothèse à l'effet que la biomasse printanière des amphipodes peut être en très étroite corrélation avec les conditions d'alimentation de l'année précédente. La signification de ce couplage pour la survie des oeufs de capelan, est étudiée.

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The availability of food and/or habitat can shape the overall seasonal cycle of abundance for many species, especially those which exploit temporally or spatially restricted resources (Tauber et al. 1986). Since Cushing's (1972, 1975) elaboration of the match/mismatch hypothesis, which proposed that spawning times of fishes should have a temporal cycle which correlates with food availability, the possible mechanisms governing these linkages have been subjected to intense study (e.g., Frank and Leggett 1986; Cushing 1990; Mackenzie 1991). However, while it is generally acknowledged that predators of the early life stages of marine fish can be abundant during the peak periods of development of the eggs and larvae of fishes (Hartig et al. 1982; Yamashita et al. 1985; Purcell 1984; DeLafontaine 1986), the process of density coupling between these predators and fish eggs and larvae has received comparatively little attention (but see Frank and Leggett 1985).

Another important gap in the literature pertaining to predation on the early life stages of marine fishes relates to the paucity of information accumulated on egg predation. Compared to studies addressing predation mortality during the larval phase (reviewed in Baily and Houde 1989), only a few studies have examined the importance of predation on fish eggs which are typically more aggregated than larvae and which should, by virtue of this aggregation, be more vulnerable to predation (Hassell 1978; Dalberg 1979; Sih 1984; McGurk 1986, 1987). The potential impact of predation during the egg stage should be particularly relevant for benthic spawners whose eggs may remain aggregated for much longer than those of pelagic spawners (Bailey and Houde 1989).

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[1] Contribution to the program of GIROQ (Groupe interuniversitaire de recherches océanographiques du Québec).

This study forms part of an in-depth analysis of predation by the Gammaridean amphipod, *Calliopius laeviusculus* on the intertidal eggs of capelin. Capelin spawning results in egg densities on the order of hundreds per cm^3 of beach sediment in the intertidal areas of thousands of bays and inlets in Newfoundland (Taggart and Nakashima 1987). Capelin egg incubation in these intertidal sediments typically extends from June to August (Frank and Leggett 1984).

The distribution of *C. laeviusculus* extends beyond that of capelin to cover most of the eastern seaboard of North America (see DeBlois and Leggett 1992 and references therein). Although *C. laeviusculus* could be classified as a trophic generalist in many of these areas, there is evidence to suggest that in Newfoundland, a close trophic link exists between this amphipod species and capelin eggs. We inspected 20 beaches located from the mouth to the head of the western coast of Conception Bay, Newfoundland, on a daily basis from May 19 to May 24 (1988), before capelin spawned, and from July 21–30 (1989), while capelin eggs developed in intertidal sediments. This visual census revealed that only those beaches where capelin spawned ($n = 10$) supported *C. laeviusculus*. Further, in a parallel study conducted at Bryant's Cove, Newfoundland, an extensively studied capelin spawning site (e.g., Delafontaine 1986; Taggart 1986; MacKenzie 1991), we concluded that *C. laeviusculus* fed almost exclusively on capelin eggs while these eggs were available and that this predation accounted for roughly half of the annual production of this amphipod (DeBlois and Leggett 1993).

In the current paper, we describe seasonal variability in the population dynamics of *C. laeviusculus* and relate these dynamics to seasonal patterns in capelin egg abundance. Targeted parameters include *C. laeviusculus* reproduction and population growth, fecundity and mortality. Our objective is to assess the hypothesis that the population dynamics of *C. laeviusculus* are intrinsically tied to the seasonal production of capelin eggs.

Materials and Methods

Sampling Design

Amphipods were sampled in the intertidal zone at a well studied capelin spawning site located at Bryant's Cove, Newfoundland. A detailed description of the study site is given in DeBlois and Leggett (1992). Quantitative amphipod samples were collected daily (except when wave height was more than 0.5 m) coincident with the daytime high tide, from May 18 to August 8, 1988. We used an epibenthic sled equipped with two 0.5 m (width) × 0.25 m (height) 350 μm nets. The sled was pushed manually (speed = 35–40 $cm \cdot s^{-1}$) over two 10 m transects located along the length of the beach. Since a large fraction of the amphipod population was found burrowed in the sediment and was therefore unavailable to our sled sampler (DeBlois and Leggett 1992), we corrected the amphipod densities obtained from this sled sampler using the relationship between densities estimated from the sled and densities obtained with a quadrat sampler which provided a more complete estimate of abundance per cm^2. Full details of the sampling procedures used and of the correction of sled density estimates are given in DeBlois and Leggett (1992).

TABLE 1. Sampling dates chosen for size and fecundity analysis. Data obtained on these dates were grouped at intervals of approximately 10 d to provide a general summary of changes in amphipod size and fecundity over the sampling season. Groups are identified throughout the text using midpoint dates.

Group	Sampling date	Group interval (days)	Midpoint date (groups ID)
1	May 29 June 4 June 8	10	June 3
2	June 12 June 16 June 21	9	June 17
3	June 25 June 29 July 5	10	June 30
4	July 8 July 12 July 18	10	July 13
5	July 23 July 25 August 1	9	July 28
6	August 4 August 8	2	August 6

Capelin eggs were sampled with 6.4 cm height × 4 cm diameter plastic corers. Three randomly placed cores were collected within each transect at 2-d intervals from June to August, 1988. Both amphipod and capelin egg samples were preserved in 5% buffered formaldehyde.

Laboratory Method

Amphipod length frequency distributions were generated for 17 sampling dates spanning the period of May–August, 1988 (Table 1). Subsamples of 100–200 amphipods were drawn from preserved samples with a Folson splitter. The body length of these amphipods (from the anterior of the head to the end of the telson), was measured from digitized images using an Optical Pattern Recognition and measuring System (OPRS). The relationship between amphipod length (mm) and dry weight (mg) was determined for 55 amphipods ranging in length from 4.5 to 15 mm. Amphipods were dried individually for 24 h at 60°C in a forced air oven.

Since sexual size dimorphism has been described for a number of amphipod species (Bousfield 1973) and could have contributed to distinct modes in size frequency distributions, we identified minimum size at maturity throughout our sampling season. Minimum size at maturity on any given sampling date was defined as the mean weight of 20 of the smallest gravid females in our samples. For males, we selected *C. laeviusculus* lacking eggs but of a size similar to that of the gravid females found in that sample. These animals were then examined for the presence of the copulatory papillae of males. We continued this visual inspection, selecting larger amphipods when necessary, until 20 males had been found. These males were then weighed. This technique for identifying sexual size dimorphism in amphipods was also used by Kevrekidis and Koukouras (1988) and Vlasblom (1969).

To predict reproductive output, the number of gravid females in each subsample was determined. Size-dependent fecundity was assessed by counting the number of eggs in the brood pouch of 33 gravid females ranging in weight from 2 to 22 mg dwt. Egg dry weight per female was obtained by drying all eggs from the brood of each of these females for 24 h at 60°C. To assess seasonal variation in egg size, we dried and weighed five replicate samples of 10 eggs taken at random from the female population on 14 sampling dates selected at 6 d intervals throughout our sampling season (May 18–August 8, 1988)

Capelin egg number in samples collected at 2–d intervals from June 17 to August 8 was estimated as follows:

$$(1)\quad \text{Egg number} = 1352 \times \text{Displacement volume (mL)}$$

$$r^2 = 0.99,\ F_{1,21} = 2388,\ p < 0.0001.$$

Capelin eggs counted for the construction of this model were then dried for 24 h at 60°C to obtain an estimate of per egg dry weight.

Data Analysis

A) Trends in Calliopius laeviusculus *and capelin egg biomass*

Amphipod biomass throughout our sampling season was defined as the product of amphipod density × average amphipod weight on any given sampling date. Capelin egg biomass was calculated as the product of egg density on any given sampling date × average egg dry weight ($0.0645\ \text{mg} \cdot \text{capelin egg}^{-1}$, SE = 0.004).

Seasonal changes in amphipod weight were estimated from length measurements using a general length–weight regression model derived from our samples:

$$(2)\quad \text{Wt} = 0.01 \times (L)^{2.66}$$

$$r^2 = 0.92,\ F_{1,55} = 621,\ p < 0.0001$$

where Wt = amphipod dry weight (milligrams) and L = amphipod length (millimetres).

Average seasonal changes in *C. laeviusculus* density are described in detail in DeBlois and Leggett (1992) and are reliably represented by the polynomial regression

$$(3)\quad D = -2.3310 + 0.1723(t) - 0.003819(t)^2 + 0.0000251(t)^3$$

where $D = \log_{10}$ density and t = days since the first sampling date (May 18, 1988).

B) Calliopius laeviusculus *size frequency distribution*

We used size frequency data, pooled by 10-d intervals (see Table 1), to document general recruitment to the amphipod population over the capelin spawning season. We employed an iterative algorithm (Press et al. 1988, section 14.4) to identify unique amphipod cohorts. This algorithm fits a pre-determined number of normal distributions to a polymodal distribution using initial estimates of the parameters (mean, standard deviation, and amplitude or number of amphipods in each distribution).

No true diagnostic test exists with which to objectively identify the number and the location of modes within a multimodal distribution (but see Hartigan 1975, for a test statistic identifying bimodal distributions when both variance estimates are equal). Size modes were therefore selected subjectively. To verify our choices, we used independently derived biological data relating to the growth rate and reproductive schedule of *C. laeviusculus* (see sections C and D below).

As a further test of our assignment of cohorts for size frequency data, we also performed a *G*-test for goodness-of-fit on models when 1, 2, and 3 Gaussian distributions were assumed. Again, it must be cautioned that *G*-tests are valid only when expected frequencies are based on hypotheses extrinsic to the data (Sokal and Rohlf 1981). While we attempted to verify our selection with independent data (described below), the *G*-test is not strictly applicable to this data. The results do however, generate insight into the goodness of fit of the distributions to the data.

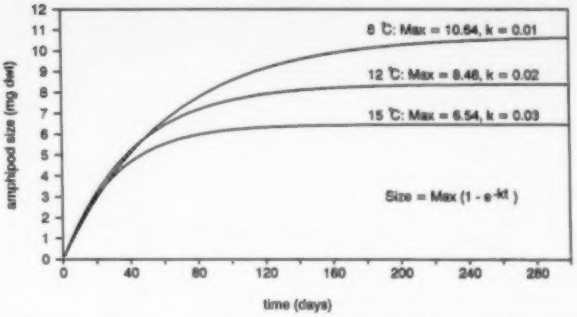

FIG. 1. *Calliopius laeviusculus* growth patterns at 8, 12, and 15 °C (from Dagg 1976). The relationships between temperature and parameters Max and *k* at these temperatures were defined and used to extrapolate growth rates over temperatures ranging from 1 to 15°C. Size specific growth rate (G_i) at these temperatures were defined from these equations (see text).

C) Calliopius laeviusculus *growth rate*

We calculated growth rates for cohorts identified using the technique outlined above (section B) and validated these using (1) growth rates observed for amphipods maintained in the lab. for a period of 42 d at 8°C, and (2) growth rates reported by Dagg (1976) for *C. laeviusculus* maintained at 8, 12, and 15°C (Fig 1). Using Dagg's data, we defined *C. laeviusculus* growth according to the Brody–Bertalanffy growth equation:

$$(4)\quad \text{Size} = \text{Max} \times (1 - e^{-Kt})$$

where Max and K are temperature sensitive parameters and t is time.

Size specific growth rates could then be generated by

$$(5)\quad G_i = 1/\Delta t \ln(\text{wt}_{t+1}/\text{wt}_t)$$

where G_i is size specific growth rate and subscripts t and $t+1$ identify weight (wt) on day t and expected weight (from eqn. 4) on day $t+1$.

D) Calliopius laeviusculus *reproductive schedule*

To calculate the release time of *C. laeviusculus* cohorts identified from size frequency data (section B) and validate our estimates with existing information on the reproductive schedule of *C. laeviusculus* in Newfoundland, we required *C. laeviusculus* growth rates applicable to temperatures of 2–4°C which prevail at our study site in spring.

Using eqn. (4) we defined the relationship between parameters K and Max and temperature at 8, 12, and 15 °C. We found that

$$(6)\quad \text{Max} = 15.36 - 0.58 \times (\text{temperature})$$

$$r^2 = 0.99,\ F = 404,\ p < 0.05$$

$$(7)\quad K = 0.002 \times (\text{temperature})$$

$$r^2 = 0.99, F = 203, p < 0.005.$$

These relationships provided an estimate of amphipod growth rates at temperatures below those tested by Dagg (1976). Since data were extrapolated, we caution that they should be viewed as approximations.

Recruitment peaks observed throughout the summer from size frequency data were compared to recruitment patterns predicted from gravid female density and fecundity observed in our samples, and from known juvenile growth rates. Recruitment patterns obtained from size frequency data were also compared with seasonal trends in amphipod abundance (eqn. 3).

Reproductive peaks were predicted from female breeding status and juvenile growth rates as follows:

The time to recruitment of juveniles in the sampled population was calculated as the time required for newly released amphipods (0.48 mm length, SE = 0.003) to grow to a length of 4 mm, at which size they became vulnerable to the sampling gear.

Density at recruitment was calculated as the product of gravid female density and fecundity on any given sampling date. A correction factor was applied to gravid female density to account for the proportion of females that would release juveniles over any sampling interval. Corrected gravid female density was calculated as

$$(8)\quad D'_b = D_b \times p\,(\text{release})$$

$$(9)\quad p\,(\text{release}) = \Delta t \times 1/\text{BDT}$$

where D'_b = corrected gravid female density, D_b = noted gravid female density and p(release) = the probability of juvenile release over any two consecutive sampling dates. Δt = the time interval in days between any two sampling dates, and BDT = 20 is average brood development time in days at noted in situ temperatures (obtained from Dagg 1976).

Juveniles density was then

$$(10)\quad D_{egg} = N_{egg} \times D'_b$$

where D_{egg} = reproductive output, N_{egg} = size specific fecundity applied to average female size on any given date, and D'_b = corrected gravid females density on this date (from eqn. 8). It should be noted that eqn. (10) assumes zero mortality from fertilization to recruitment. Juvenile mortality is discussed below (Section E).

E) Seasonal variation in C. laeviusculus *fecundity and mortality*

The influence of female size and sampling date on egg weight were assessed independently through least squares regression. *Calliopius laeviusculus* mortality was calculated separately for juveniles and adults sampled from May 18 to August 8, 1988. Overwintering mortality was estimated for adults only.

To estimate juvenile mortality, we compared predicted density at recruitment from eqn. (10) with the observed recruitment of 4 mm (length) individuals. Mortality was calculated as

$$(11)\quad M = 1/\Delta t \times \ln(D_4/D_{egg} \times S)$$

where M = mortality (per day), D_4 is the sum over all sampling dates of the density of amphipods 4 mm in length observed in our samples, D_{egg} is the sum of the estimated reproductive output from June 3 to August 6 (from eqn. 10), $S = 0.75$ is amphipod brood survival estimated by Moore (1981) and $\Delta t = 8$ is the average predicted time (days) from brood release to recruitment at observed in situ temperatures.

Instantaneous mortality (M) of adults sampled over the summer was calculated during a period of low recruitment (June 29 to July 26) as

$$(12)\quad M = 1/\Delta t \times \ln(D2/D1)$$

where M = mortality (per day), $D1$ and $D2$ are average amphipod densities (from eqn. 3) on June 29 and July 26, respectively, and $t = 27$ is the interval between these two dates.

Overwintering mortality of adults was approximated using our data on spring and fall amphipod densities, plus the expected reproductive output from females sampled in the fall. We made the simplifying (and necessary) assumption that the density of the spring and fall population in 1988 gave an average estimate of amphipod densities at these times in any given year. This assumes relatively stable variation in population size from year to year. If, on the other hand, *C. laeviusculus* density exhibits substantial interannual fluctuations, these calculations would not produce reliable results.

Overwintering mortality was calculated using eqn. 12 but in this case M = overwintering mortality, $D1$ = amphipod density noted on the first sampling date (May 18), $D2$ = amphipod density noted on the last sampling date (August 8) plus the expected reproductive output from females sampled on that date, and $t = 276$ is the time interval in days between August 15 (the time of full recruitment for juveniles released after August 8) and May 18.

Results

Trends in *Calliopius laeviusculus* and Capelin Egg Biomass

Average changes in the median size of *C. laeviusculus* from May 18 to August 8, 1988 were described by

$$(13)\quad L = 9.1495 - 0.02812(t) + 0.009103(t)^2 - 0.00007350(t)^3$$

$$R^2 = 0.57, F_{3,72} = 31.61, p < 0.0001$$

where L = median amphipod length and t = days since the first sampling date.

Seasonal changes in amphipod density (eqn. (3); Methods) and size are shown in Fig. 2. Density peaks observed on June 29 and August 8 each co-occur with size minima which suggests high recruitment of small amphipods at these times.

Capelin spawned three times in 1988 with peak egg deposition occurring on June 23, June 29, and July 11 (Fig. 3). Amphipod biomass closely tracked capelin egg biomass throughout the capelin egg development period. In August, amphipod and capelin egg biomass became decoupled and amphipod biomass increased in the absence of capelin eggs.

Calliopius laeviusculus Size Frequency Distribution

Seasonal variation in the size distribution of amphipods was substantial (Fig. 4). Three distinct cohorts were identifiable on June 3. These cohorts were obvious until July 13. On July 13, a forth cohort was identified in the sampled population. Distinct cohorts were not readily identifiable on July 28 or on August 6. Recruitment was, however, high on these two dates. The highest recruitment levels were noted on August 6 when the population was dominated by small individuals. We postulate the appearance of a fifth cohort on this date.

A summary of mean and standard deviation statistics when 1, 2, and 3 Gaussian distributions were assumed for these size frequency data is provided in Table 2. A G value smaller than

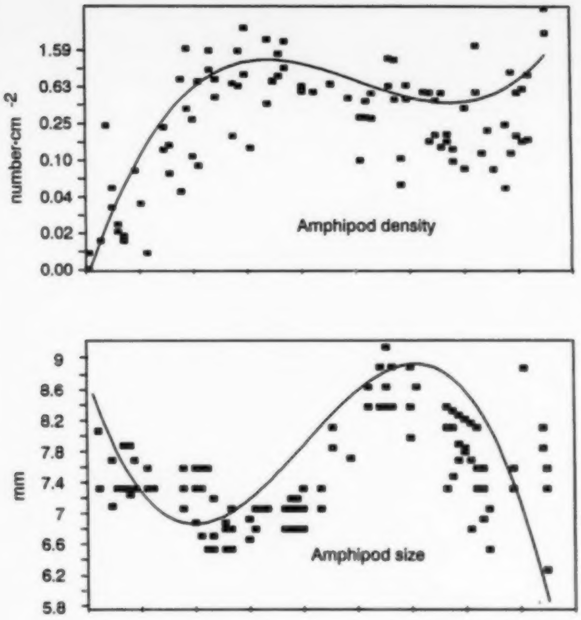

FIG. 2. Density and size of *C. laeviusculus* at Bryant's Cove (Nfld.) collected from May to August, 1988. Density $(D) = -2.3310 + 0.1723(t) - 0.003819(t)^2 + 0.0000251(t)^3$, $R^2 = 0.56$, $F_{3,92} = 38.63$, $p < 0.0001$ (DeBlois and Leggett 1992); where D is $\log_{10}$ density and t is time since our first sampling date (May 18, 1988.). Size $(L) = 9.1495 - 0.02812(t) + 0.00913(t)^2 - 0.00007350(t)^3$, $R^2 = 0.57$, $F_{3,72} = 31.61$, $p < 0.0001$; where L is median amphipod length and t is time since our first sampling date. Logarithmic axes (amphipod density) were backtransformed to arithmetic values.

$\chi^2 = 37.65$ indicates that the data do not deviate significantly from the fitted distribution. In most cases, a combination of 3 Gaussian distributions provided a good description of the data.

Calliopius laeviusculus Growth Rate and Reproduction

Sexual size dimorphism can not account for cohorts identified from June 3 through August 6, 1988. Figure 5 indicates that sexual size dimorphism (males > females) is only prevalent in individuals > 9 mm in length. These larger individuals were part of the overwintering population and were grouped into cohort I. Cohort I accounted for only ca. 1% of the sampled population on June 3. Sexual size dimorphism gradually disappeared over the summer months as these overwintering animals disappeared and cohorts II and III began to dominate the reproductive population.

Given a knowledge of in situ temperatures during the development of juveniles, and of temperature and size sensitive amphipod growth rates, approximate release times can be calculated for cohorts tentatively identified in Fig. 4.

Yearly temperature variation in the intertidal at Bryant's Cove was approximated from published temperature data recorded at a nearby beach (see Fig. 6). Growth functions at temperatures ranging from 2 to 15 °C were generated from Dagg (1976) (see Methods, section D). Growth rates generated from these functions were in agreement with growth rates observed in the field and in the laboratory ($t = 0.179$, $p > 0.1$) (Table 3).

Analysis of these amphipod growth functions in relation to

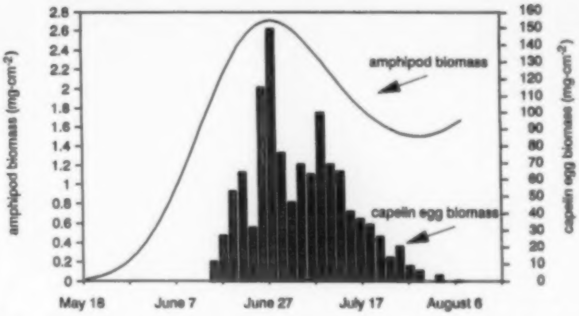

FIG. 3. Biomass of capelin (*Mallotus villosus*) eggs and amphipods (*C. laeviusculus*) at Bryant's Cove (Nfld.) from May to August, in 1988. *C. laeviusculus* biomass was derived from functions given in Fig. 2.

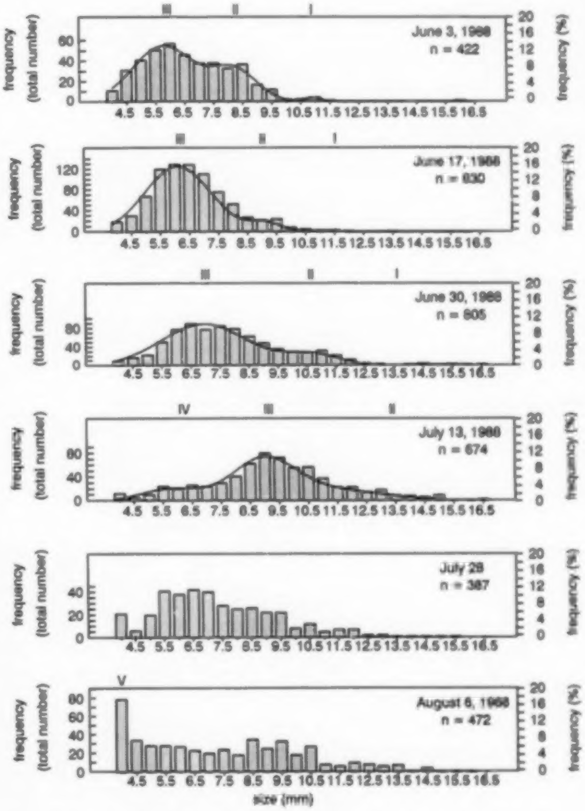

FIG. 4. Population size structure of *C. laeviusculus* at Bryant's Cove (Nfld.) from June to August, 1988. Amphipod cohorts I to V are shown. Distributions were fitted using an algorithm derived by Press et al. (1988) (see text).

in situ temperatures, indicated that amphipods released before December in a normal year should achieve an asymptotic size of ca. 11 mm (length) by June of the following year. We identified

TABLE 2. Summary of mean and standard deviation statistics for size frequency data fitted with 1, 2, and 3 Gaussian distributions. *G* test (df = 25) results are given for each trial. *G* values $\chi_{[0.05]}^2 = 37.65$ indicate that data do not deviate significantly from the fitted distributions.

Date	No. of Gaussian distributions	X1	X2	X3	STD1	STD2	STD3	N1	N2	N3	*G*
	I	6.36			1.80			422			20.10
June 3	II	5.79	8.26		1.08	0.80		296			46.32
	III	5.80	8.27	10.97	1.09	0.79	0.32	294	122	6	2.00
	I	6.13			1.21			830			234.82
June 17	II	6.14	8.00		1.03	1.51		634	196		11.56
	III	6.29	9.35	11.39	1.14	0.59	0.45	764	56	10	3.65
	I	7.37			1.86			805			301.28
June 30	II	7.14	10.88		1.50	0.99		678	127		226.67
	III	7.15	10.89	15.19	1.50	0.95	1.05	670	119	16	4.85
	I	9.28			1.91			674			226.94
July 13	II	5.62	9.42		0.84	1.59		75	599		281.94
	III	6.29	9.35	11.39	1.14	0.59	0.45	112	320	243	37.18

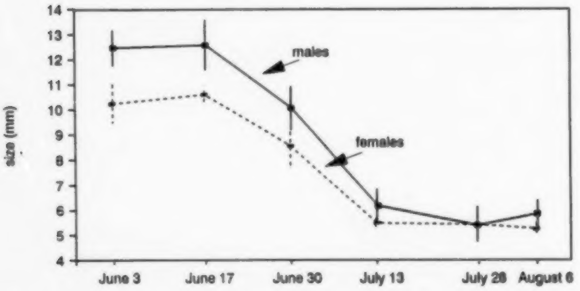

FIG. 5. Minimum length at maturity for *C. laeviusculus* sampled at Bryant's Cove (Nfld.) from June to August, 1988. Values reported are the average (±1 SE) of measurements taken on 20 individuals (see text).

these individuals as members of cohort I (Fig. 4). Mean amphipod length for cohorts II and III on June 3 were 8.27 and 5.80 mm, respectively. Based on growth rates predicted for the March to June interval, we estimated that cohort II was released in April and that cohort III was released near the end of May.

Size frequency data (Fig. 4) suggest that two additional cohorts were released during the summer, one in early to mid July and the second in early August. Seasonal variations in amphipod density (Fig. 2) also indicate two recruitment intervals over the summer, centered on June 29 and August 8.

These observations are consistent with observed gravid female density and predicted juvenile recruitment throughout our sampling period. From data on gravid female density, female size at maturity (Fig. 5), size dependent fecundity (Fig. 7) and growth rates for juvenile amphipods at in situ temperatures, we predicted high recruitment in late June and again in early August. Gravid female density and predicted reproductive output throughout the sampling season is shown in Fig. 8.

Seasonal Trends in *Calliopius laeviusculus* Fecundity and Mortality

Although we noted seasonal differences in female size (Fig. 5), we found no relationship between egg weight and female weight ($F_{1,31} = 1.20$, $r^2 = 0.08$, $p = 0.29$) for amphipods ranging in weight from 2.5 to 20 mg dwt. There was also no

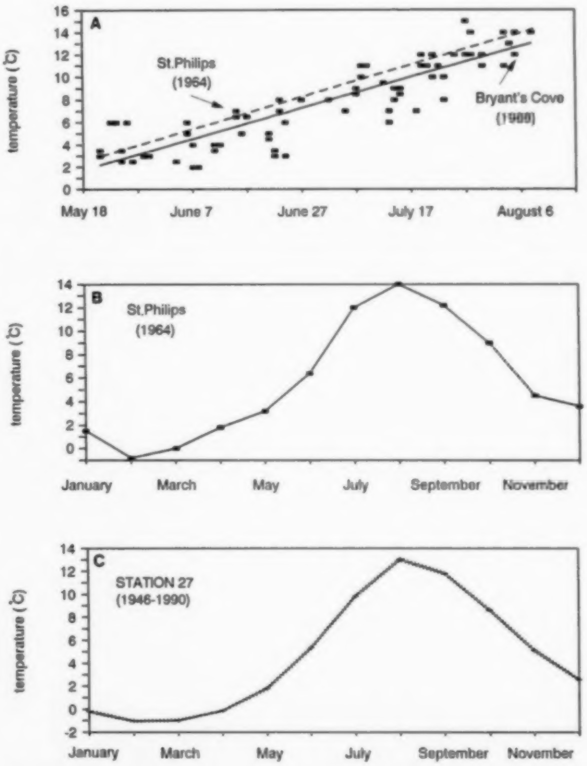

FIG. 6. (A) Summer sea temperature profiles (May–August) recorded at the low tide zone at Bryant's Cove (1988) and St. Philips (1964) (Steele and Steele 1970a), Nfld. (B) Annual sea temperature profile recorded at St. Philips, Nfld., in 1964 (Steele and Steele 1970a). (C) Long-term surface temperature record, including 95% CI, recorded at hydrographic station 27, St John's, Nfld. (B. DeYoung, Memorial University, personal communication). Temperatures recorded from May to August at Bryant's Cove (1988) and St. Philips (1964) did not differ significantly ($t_{\mathrm{slope}} = 0.22$, $p > 0.5$, $t_{\mathrm{intercept}} = 1.55$, $p > 0.5$). A comparison of data from station 27 with 1964 data from St. Philips indicates that this year was not aberrant relative to long-term means.

TABLE 3. Instantaneous growth rates for *Calliopius laeviusculus* estimated from field and laboratory studies.

Cohort	Δt (days)	Temperature (°C)	Initial size (mg)	Observed growth ($mg \cdot d^{-1}$)	Expected growth ($mg \cdot d^{-1}$)
III	14	4	1.07	0.016	0.048
III	13	6	1.33	0.026	0.048
III	14	8	1.87	0.053	0.038
II	14	4	2.76	0.024	0.018
II	13	6	3.82	0.032	0.014
II	14	8	5.73	0.009	0.006
I	14	4	5.84	0.007	0.008
I	13	6	6.46	0.060	0.005
Lab. animals	42	8	0.48	0.054	0.053

TABLE 4. Mortality estimates for amphipod juveniles, adults (summer), and overwintering adults.

	*D*1 ($no. \cdot cm^{-1}$)	*D*2 ($no. \cdot cm^{-1}$)	Interval	Δt (days)	*M* ($no. \cdot cm^{-1} \cdot d^{-1}$)
Juveniles	6.87	0.460	Release – recruitment	8	0.34
Adults	1.09	0.450	June 29 – July 26	27	0.03
Overwintering adults	0.81[a]	0.046	August 15 – May 18	276	0.01

[a] *D*1 on August 15 was calculated from observed amphipod density on August 8 (0.66 amphipods $\cdot$ cm^{-2}) and extrapolated to August 15 using an adult mortality of $M = 0.03 \cdot d^{-1}$, plus the total density of juveniles expected to be released from August 8 to 15 (0.15 amphipods $\cdot$ cm^{-2}) based on gravid female density and juvenile mortality.

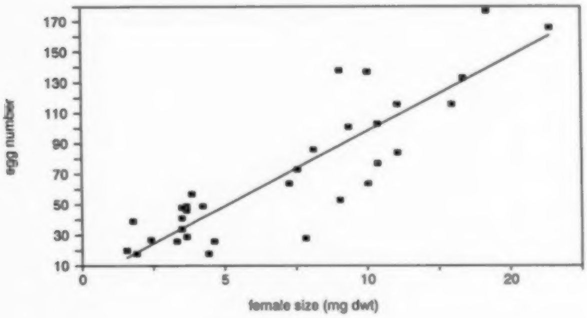

FIG. 7. Relationship between female size (mg dwt) and fecundity for *C. laeviusculus* at Bryant's Cove, Nfld., in 1988. Egg number = 8.085(mg dwt), $r^2 = 0.94$, $F_{1,31} = 445$, $p < 0.0001$. Average amphipod egg weight was 0.02 mg (dwt) (SE = 0.006).

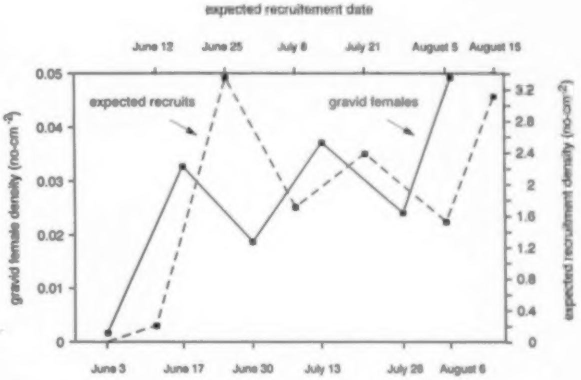

FIG. 8. Gravid female density and expected recruitment for *C. laeviusculus* at Bryant's Cove, Nfld, from June to August, 1988. Recruitment was calculated from estimates of brood development time and the development time of juveniles to recruitment. Recruitment estimates do not include juvenile mortality from brood release to recruitment (see text).

relationship between egg weight and sampling date ($F_{1,13} = 0.67$, $r^2 = 0.053$, $p = 0.43$).

Instantaneous mortality estimates (amphipods $\cdot$ d^{-1}) for juveniles and adults sampled in the spring and summer, and for overwintering adults, are given in Table 4 together with input variables for each calculation. Mortality for juveniles, adults (summer), and overwintering adults were 0.34, 0.03, and 0.01 amphipods $\cdot$ d^{-1} respectively.

Discussion

An important assumption underlying the work described above and the conclusions which follow is that our study system is more or less closed to *C. laeviusculus* migration from April to August. The available evidence supports this assumption: First, in Newfoundland, *C. laeviusculus* is not found on beaches where capelin do not spawn. The absence of *C. laeviusculus* on these beaches both in May, prior capelin spawning, and again in July, after eggs had been deposited on a number of spawning beaches, suggests that small scale migrations are uncommon, otherwise vagrants would regularly occur on beaches lacking capelin eggs. Secondly, DeBlois and Leggett (1993) have shown that the energetic requirements of *C. laeviusculus* are met on capelin spawning beaches from early spring to August. The capelin egg food source is particularly rich and provides more than ideal conditions for growth to *C. laeviusculus* during the summer months (DeBlois and Leggett 1991, 1993). From general theories on animal behavior (e.g., Krebs 1978), we do not expect animals to migrate out of an area where food is plentiful (but see DeBlois and Leggett 1993, on the fall migration of *C. laeviusculus* following the disappearance of capelin eggs). Finally, the amphipod population growth observed in 1988 can be accounted for solely on the basis of reproduction. Further, the similarity in growth rates for cohorts sampled in the field to growth rates obtained for individuals maintained under laboratory conditions suggests that little migration occurs. If, for example, a large number of animals in a given size class entered our population from another source, size frequency data would become skewed toward this size class and calculations of growth rates would diverge from expectations.

Calliopius laeviusculus biomass closely tracked capelin egg biomass throughout the capelin egg development period. While our sampling methods did not capture amphipods smaller than 4 mm in length, these animals, owing to their small size, comprised only a small fraction of the total population biomass (We estimate < 10% of the total amphipod biomass from May to August, 1988; see DeBlois and Leggett 1993). In addition, newly released juveniles (0.48 mm) would require only 8 days to reach a size of 4 mm at in situ temperatures. For these reasons, we conclude that the incorporation of biomass estimates for animals <4 mm into our global biomass estimates would not significantly alter the trend observed in seasonal amphipod biomass.

The first major peak in amphipod biomass occurred in late June and resulted from high amphipod reproductive activity in late April and again at the end of May. The interval between these two reproductive bouts matched brood development times expected at in situ temperatures observed in May (Dagg 1976). Juveniles released during each of these two reproductive periods formed two cohorts that recruited into the sampled population between May and the end of June. A lull in amphipod reproductive activity during June lead to low recruitment and decreasing biomass during July. Amphipods from cohorts II and III did not become reproductively active until mid July. Young released by cohorts II and III entered the sampled population in August and resulted in a second peak in amphipod biomass.

These patterns in *C. laeviusculus* life history are in agreement with those reported by Steele and Steele (1973) who studied this species at St. Philips, Conception Bay, Newfoundland. They found that at St. Philips *C. laeviusculus* had a semiannual life cycle; that the overwintering population released juveniles from March to June; that these overwintering animals were larger than adults present in the summer; and that juveniles released in the spring matured and reproduced before fall.

While capelin do spawn at the beach at St. Philips, egg deposition is typically much lower than that noted at Bryant's Cove (Nakashima, B., Department of Fisheries and Oceans, St John's, Nfld., personal communication, DeBlois, E.M., pers. obs.). Since Steele and Steele (1973) did not quantitatively measure amphipod abundance at their study site, we cannot make a direct comparison of amphipod biomass between sites.

Calliopius laeviusculus population size peaked during the period of maximum capelin egg abundance on Bryant's Cove. However, we found no evidence of a within season causal relationship between amphipod and capelin egg abundance. Had the proximal cause of increases in amphipod biomass at Bryant's Cove been predation on capelin eggs, the amphipod population should have peaked subsequent to, and not coincident with, the peak in capelin egg abundance.

Females of *C. laeviusculus*, like those of a number of intertidal gammarideans found at these latitude (e.g., Steele and Steele 1970a, b, 1973, 1981), exhibit a resting stage from August to November. The initiation and termination of this reproductive diapause has been attributed to temperature, photoperiod, food availability, or a combination of these (Powell and Moore 1991 and reference therein). The functional significance of this sexual quiescence is linked to the need to release post-emergent hatchlings at the most appropriate time for successful foraging when the growing season is limited (Wildish 1982).

In 1988, two *C. laeviusculus* cohorts were released at least 18 d prior to capelin spawning and the resultant increase in food abundance in the intertidal community. Given that water temperatures were <5°C prior to capelin spawning, amphipod growth and metabolic demands were low (DeBlois and Leggett 1993). Capelin typically begin spawning in early to late June (Frank and Leggett 1981, 1983, 1984) when water temperature begins to rise (see Fig. 6). In most years the energetic demand of the *C. laeviusculus* population is likely to remain low until capelin spawning is initiated.

As noted previously, the initial peak in *C. laeviusculus* biomass was not a direct response to capelin egg biomass. However, the onset of *C. laeviusculus* reproduction in spring, coupled with increased temperatures which usually coincide with capelin spawning, could promote an overall coupling between *C. laeviusculus* and capelin egg abundance.

A true numerical response by the *C. laeviusculus* population to capelin egg consumption appears to have occurred in August when amphipod biomass increased to a second maximum. Capelin eggs were, by far, the dominant food in the intertidal area of Bryant's Cove from June to August 1988 (DeBlois and Leggett 1993). Since most of the development and growth of cohorts II and III overlapped this high capelin egg abundance, the reproductive output of these two cohorts, which appeared as juveniles in samples collected in early August, can be attributed to the good feeding conditions experienced by their parents. Several authors have previously noted high levels of amphipod reproduction when food is abundant (e.g., Powell and Moore 1991, Steele et al. 1977, Segerstrale 1967).

We found no evidence that amphipod fecundity or mortality responded to changes in capelin egg density. The fecundity of individual females was higher earlier in the summer owing to larger female size. Egg weight was unaffected by female weight or sampling date, although Steele and Steele (1973) did find that the eggs of *C. laeviusculus* sampled during the winter were larger than those from amphipods sampled in summer. Our sampling interval did not cover the winter months.

Amphipod mortality was estimated to have been lower during winter ($M = 0.01 \cdot d^{-1}$) than during summer ($M = 0.03 \cdot d^{-1}$). This difference was also noted by Steele (1964). Because average amphipod size is larger in winter, it is difficult to decouple the possible influence of seasonality on mortality from the reduction in mortality known to occur with increasing size (Wildish and Peer 1981, Skadsheim 1990.)

Few studies report estimates of amphipod mortality. From survivorship curves for *Pontoporeia femorata* reported in Wildish and Peer (1981), we calculated an average annual adult mortality of $0.016 \cdot d^{-1}$. Skadsheim (1990) reported a mortality rate for laboratory-reared *Gammarus salinus* of $M = 0.11 \cdot d^{-1}$ during the first week after brood release and an average mortality of $M = 0.02 \cdot d^{-1}$ for subsequent stages. Our estimates of adult mortality were consistent with these studies. However, our estimate of juvenile mortality, derived from field samples, was much higher ($M = 0.34 \cdot d^{-1}$) than Skadsheim's laboratory estimate.

Our data are consistent with the hypothesis that *C. laeviusculus* populations in Newfoundland are maintained by the annual reoccurrence of high abundances of capelin eggs in the intertidal at numerous, but selected, sites along the coast. Endogenous reproductive patterns influenced by exogenous seasonal stimuli could allow *C. laeviusculus* biomass to closely track capelin egg biomass thus providing favorable feeding conditions during most of the more active life phases of this species.

Further, the reproductive diapause noted in this species, coupled with relatively constant overwintering mortality, could serve to effectively link *C. laeviusculus* population size to feeding conditions experienced in the previous season. At present, we have no information on the interannual variation in overwintering mortality for *C. laeviusculus*. Van de Veer and Bergman (1987), studying *Crangon crangon* (Crustacea: decapoda) predation on 0-group plaice in intertidal nursery areas of the Dutch Wadden Sea, found that overwintering mortality in *C. crangon* was relatively stable and that only extremely cold winters resulted in unusually low numbers in spring. If a similar scenario occurs for *C. laeviusculus* in Newfoundland, then a series of years in which capelin egg biomass was high could lead to a gradual increase in amphipod biomass over the spawning bed and a corresponding increase in predation pressure on capelin eggs.

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Influence of Calcium Concentrations on Cadmium Uptake by the Freshwater Mussel *Elliptio complanata*

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Wang, Y., and R.D. Evans. 1993. Influence of calcium concentrations on cadmium uptake by the freshwater mussel *Elliptio complanata*. Can. J. Fish. Aquat. Sci. 50: 2591–2596.

The effect of calcium ions on the uptake of cadmium by the freshwater mussel *Elliptio complanata* was studied in controlled laboratory experiments. Radioactive ^{45}Ca and ^{109}Cd were used as tracers. The experiments were designed to minimize geochemical differences between treatments so that the physiological interactions of the two ions could be studied. Uptake of Cd was correlated with concentration of Cd in the experimental tanks. However, the concentrations of Ca also had a strong impact on uptake rates. When uptake of Cd was expressed as a function of the molar ratio of Cd to Ca, greater than 96% of the variation could be explained.

Au cours d'expériences en laboratoire, on a étudié l'effet des ions calcium sur l'absorption du cadmium chez l'anodonte *Elliptio complanata*. On a utilisé du ^{45}Ca et du ^{109}Cd radioactifs comme marqueurs. Les expériences ont été faites de manière à minimiser les écarts géochimiques entre les traitements de façon à ce qu'on puisse étudier les interactions physiologiques des deux ions. On a établi la corrélation entre l'absorption de Cd et la concentration de Cd dans les bassins qui servaient aux expériences. Cependant, la concentration de Ca a un effet marqué sur les taux d'absorption. Lorsque l'absorption de Cd est exprimée en fonction de la proportion molaire de Cd par rapport au Ca, plus de 96 % de la variation peut être expliquée.

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Accumulation of cadmium (Cd) by aquatic organisms can occur either via direct uptake across body membranes or via indirect uptake by absorption from digested food in the gut tract. The relative importance of these two uptake mechanisms remains open to debate and probably varies among organisms (Abel and Bärlocher 1988; Evans and Lasenby 1993).

Of the two processes, direct uptake has been the more difficult to quantify. Direct uptake involves the transport of ionic or complexed cadmium to receptor sites, followed by transfer through an animal's membrane. In most cases, only the free metal ion is expected to interact with the binding site and the extent of binding would depend on the ion activity. Thus it is generally believed that the ionic form (aquo-ion) of most metals, including Cd, is most available to aquatic organisms (Stokes and Campbell 1985; Morel and Morel-Laurens 1981) and that the total Cd concentration in water does not provide a reliable indication of bioavailability (Laxen 1984). Variation in the activity of the free metal ion will affect the rate of its uptake. Many physicochemical factors such as redox potential, pH, sorption, chelation, complexation, precipitation, and hydrolysis may affect water Cd speciation, and therefore activity of the free ionic form.

For freshwater mussels, gills are proposed as the major site of metal uptake from solution, because of their large surface area and the immediate accumulation of any administered dose (Holwerda et al. 1989). It has been suggested that 90% of Cd uptake is by absorption from solution, and is facilitated by diffusion of $CdCl_2$ across the gills or by some type of complexation with a high molecular weight compound present on the gill surface (Carpene and George 1981).

Cadmium has no known metabolic functions and our knowledge about its transport mechanism at the molecular level across cell membranes is still very limited. Most information comes from the study of gill transport in fish (e.g., Wicklund and Runn 1988). Proteins are believed to be the pathway of substances between the intra- and extra-cellular compartments. Possible mechanisms include: passive co-diffusion of polar ions through pores along a potential gradient (Stein 1967); complexation with ligands normally used to transport nutritionally essential cations (Hunn 1985); and complexation of specific ligands with metal ions which induces a conformation change in the membrane (Pärt et al. 1985). A better membrane transport model is required to assess the toxicological and environmental importance of Cd.

It has been reported that water hardness inhibits Cd uptake in many freshwater organisms (Wicklund and Runn 1988; Abel and Bärlocher 1988). How this happens is unknown. There are two possible mechanisms by which major cations such as Ca and Mg could affect Cd uptake. At the geochemical level, major cations can compete for binding sites on organic and inorganic ligands causing an increase in Cd^{2+} activity (Campbell and Evans 1987). At the organism level, it has been suggested that Ca can act directly in one of several ways, thereby depressing the heavy-metal uptake. Competition for binding sites on the membrane between Ca and Cd ions could alter Cd uptake (Hunn 1985), as could gill permeability changes induced by altered Ca concentrations (Rogers and Beamish 1983; Pärt et al. 1985). Of these hypotheses, the competition mechanism is the most popular and widely accepted. Stated simply, the process involves the accidental uptake of Cd across the membrane through Ca uptake channels.

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There is some evidence to support the competition mechanism. For example, Cd^{2+} can be substituted for Ca^{2+} on Ca-binding proteins (Suzuki et al. 1985; Flik et al. 1987). Also, Nilsson et al. (1986) suggested that Cd and Ca utilize a similar transport mechanism in pancreatic Beta-cells.

Identification of the potential for Ca control of Cd uptake is important from an environmental management perspective. If Cd uptake by aquatic invertebrates is largely controlled by available Ca concentrations in lakewater, then we should be able to predict those systems in which we would expect elevated body burdens of Cd in organisms. If, on the other hand, the observed effects of the presence of Ca on Cd burdens in organisms (Campbell and Evans 1991) is largely a geochemical phenomenon, then prediction of organisms' concentrations will be much more dependent on subtle geochemical differences between systems and prediction will be much more difficult.

In this study, we have examined the role of Ca in Cd uptake in a controlled laboratory experiment using the freshwater mussel *Elliptio complanata*. This approach allows us to separate geochemical and physiological influences and to examine more precisely the role of Ca in Cd uptake across the gill membrane.

Methods

Over 1000 mussels were collected from a sandy sediment area (water depth ~2–3.5 m on the south shore of Deer Bay, Harvey Township, Peterborough County, Ontario (Longitude 78°26′ Latitude 44°28′) during the late summer of 1988. This site was chosen because both the Otonabee River (which provided the water for the laboratory experiments) and Deer Bay are located on the Trent Waterway system, and therefore, have similar water chemistry characteristics (Table 1).

The mussels were chosen by shell length in order to control size variation as much as possible (±1.5 cm), because mussel size has a strong negative correlation with trace metal burdens. Jones and Walker (1979) found that larger mussels (with heavier tissue weights) from the same area, regardless of age and sex, had lower metal concentrations than did smaller mussels (with lower tissue weight). Since shell length of mussels is positively correlated with tissue weight (Fischer 1988), variation in shell length (as the only measurable parameter when a mussel is alive) was minimized to eliminate variation in tissue weight and perhaps metal uptake.

Preliminary experiments (Y. Wang and R.D. Evans, unpublished data) revealed that the mussel shells were scavengers of most of the free Cd ions in the water. More than 90% of Cd lost from water was adsorbed onto the shells. Thus, a problem arose in trying to maintain a stable Cd concentration in the water throughout an experiment. To counteract this problem, before the experiment started, the shells of all mussels were stripped of their periostracum and any attached mosses, and polished to provide reasonably smooth surfaces. They then were coated with two layers of acrylic nail polish. After the shells had been coated, these mussels were left sitting in 0.45 μm filtered water for 2 d to evacuate their guts, preventing adsorption of Cd from the water by feces excreted during the experimental period. This treatment had no apparent effect on the organisms as survival post treatment of at least 2 yr in tanks has been observed. Mortality during the experiment was zero. The treatment containers were large plastic pails which had a water volume of 19 liters. Preliminary experiments indicated that this volume was necessary to ensure that during the experiment Cd and Ca concentrations in the water remained at a level not less than 90% of the initial concentrations (Fig. 1).

The radioisotopes ^{109}Cd and ^{45}Ca were used as tracers for the uptake of stable Cd and Ca in mussel tissue. Specific activity was varied by adjusting stable Cd and Ca concentrations. Knowledge of the specific activity of each element in the organisms, at the outset and at the conclusion of an experiment, allows calculation of the uptake rates of the stable elements. Cadmium concentrations ranging from 4 to 120 μg/L and Ca concentrations ranging from 43 to 130 mg/L were used. A five by five matrix design was employed to produce 25 different ratios of Cd to Ca. The experiment was repeated using a smaller number of ratios, resulting in a total of 31 different ratios of Cd to Ca ranging from 0.04 to 2.7 μg Cd/mg Ca. In addition, one control tank containing five mussels but no radioisotopes or stable Cd or Ca was used to monitor Mg, Cl, O_2, temperature, conductivity, and pH throughout the 72 h experiment.

Otonabee river water (Table 1) was filtered through a polypure 60 μm and 0.45 μm double filter system prior to use in the experiments. Stable $CdCl_2 \cdot 2\frac{1}{2}H_2O$ (Mallinckrodt) was used for its high solubility ($\log K_{sp} = 2.6$) producing mostly Cd^{2+} which should be the most bioavailable chemical form of this element. Changes in Cl^- concentration from lowest to highest treatment from addition of $CdCl_2$ were less than 1% of the total concentration and less than analytical variability in Cl measurements. Thus it is unlikely that changing Cl concentrations throughout the treatments affected uptake of Cd (Jackim et al. 1977; Fischer 1986). Because much higher concentrations of Ca were added, stable $Ca(NO_3)_2 \cdot 4H_2O$ (Fisher Scientific) was used to alter Ca concentrations. The addition of nitrate to the tanks should not affect Cd uptake. Each tank contained ^{109}Cd and ^{45}Ca activities of 35 and 1005 Bq/mL, respectively.

Preliminary experiments (Wang 1990) indicated that aeration was necessary to maintain dissolved oxygen levels and to prevent the formation of a concentration gradient within the tanks. Since Cd speciation is pH dependant, pH and conductivity were closely monitored as well. Concentrations of Cd, Ca, Cl, and Mg as well as dissolved O_2, pH, temperature, and conductivity were measured initially in all tanks. Water samples were taken at 12-h intervals from each tank for Cd and Ca while samples from the non-radioactive control tank only were measured for all other parameters at the same intervals. Ca and Mg concentrations were measured by flame atomic absorption spectrophotometry and Cd concentration was performed by Zeeman flameless atomic absorption spectrophotometry. Water samples for these elements were kept in polyethylene vials at 4°C until analyzed. Cl was determined using an ion specific electrode, oxygen using a dissolved oxygen meter, pH using a pH meter and conductivity and temperature using a combined meter. All of these parameters were measured in situ.

Five randomly selected mussels were placed in each of the 25 treatment tanks for 72 h, following which they were removed and killed. The soft tissues were removed from the shell, dried at 90°C for 24 h then ground into powder before further analysis. Shells were air dried and their weights and lengths were measured. Radioactivity was measured in each of the five organisms from each tank.

Activity of ^{45}Ca was measured by liquid scintillation counting (Beckman LS7000). A 2-mL water sample was mixed with

TABLE 1. Water chemistry for Otonabee river water used in experimental tanks and Deer Bay from which mussels were collected. All measurements have units of mg/L except for Cd which has units of μg/L. Values from this study for Otonabee water indicate mean and standard deviation for measurements taken during the 72 h of the experiment in the control tank containing five mussels but with no radioactive or stable Cd or Ca added.

	Otonabee River	Deer Bay[d]
Calcium	30.0 ± 2.06	26.5
Cadmium	0.2 (D.L.)[a]	0.02
Chloride	7.5 ± 0.5	
Magnesium	4.0 ± 1.12	
pH	7.9 ± 0.25	7.5
conductivity	190 ± 10.7	
oxygen	9.8 ± 1.3	9.0
temperature	22 ± 1	21[e]
Sodium	3.7[b]	
Potassium	0.7[b]	
Sulphate	7.9[b]	
Nitrate	0.01[c]	

[a] Detection Limit.

[b] Measured by Ontario Ministry of Environment, 1987.

[c] Measured by Ontario Ministry of Environment, 1976.

[d] From Campbell (1987).

[e] Measured at the same time of year as the present study.

18 mL of Aqueous Counting Scintillant (ACS-Amersham), and allowed to sit in complete darkness for 48 h in order to quench light excitation prior to counting. Ground, dry tissue (0.1 g) was dampened with 0.2 mL of distilled water followed by 2 mL of NCS tissue solubilizer (quaternary ammonium base in toluene, Amersham). The capped vials were kept in a 50°C sand bath for 24 h or until all of the tissue was digested. Finally, 10 mL of Organic Counting Scintillant (OCS, Amersham) was introduced. All samples were corrected for color quenching using Nitromethane (CH_3NO_2) colour standards. Activities of ^{109}Cd in the water and tissue samples were determined by gamma spectroscopy using a well-type NaI detector. Aliquots of 0.5 mL of water and 0.2 g of tissue were counted in a uniform geometry with no prior sample preparation. Preliminary tests indicated that there was no interaction between the Cd and Ca isotopes in either counting procedure.

Results and Discussion

Many factors other than available ion concentrations can affect both the geochemistry of a system and the physiology of the organisms, and thus ultimately affect trace metal uptake rates. Many of the geochemical variables were eliminated by the experimental design. For example, the same minimal concentration of dissolved organic carbon (DOC ~1 mg/L, R.D. Evans, unpublished data) was present in each experimental tank. Preliminary experiments were used to determine the most important variables which might alter organism physiology during the experiments. These were found to be water temperature, pH, dissolved oxygen (DO), and Mg concentrations (Wang 1990). Because of the large volume of water relative to the number of mussels, these parameters did not vary significantly over the course of the experiments (Table 1).

The Ca and the Cd were added as compounds chosen to keep the largest amounts as the free ionic forms. The chemical speciation model MinteqA2 (version 3.11, available from the U.S. E.P.A. Centre for Exposure Assessment Modeling, Athens, Georgia) was used to calculate the theoretical speciation in each experimental container. One limitation to these calculations is that the model (like all equilibrium models) does not have good partition coefficients for DOC. Since DOC concentrations were low, the estimates of free metal concentrations will be only slightly high. Free Ca^{2+} was constant at 97.5% ± 0.2 of total concentration (independent of concentration) while the percentage of total Cd predicted to be in the free Cd^{2+} form varied between 40.6 and 46.0%. The proportion of free Cd increases with increasing Ca concentration primarily due to competition for the carbonate ligand to which most of the remaining Cd is bound.

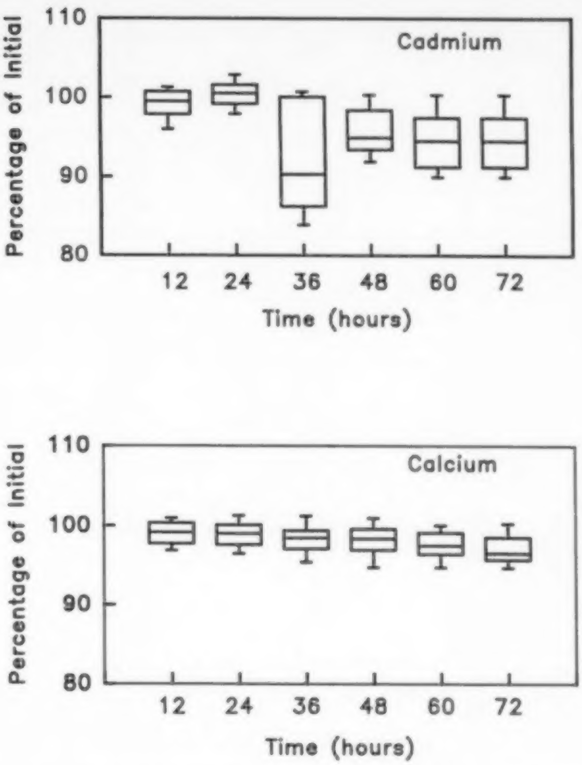

FIG. 1. Changes in Cd and Ca concentration through time expressed as a percentage of the initial concentration. Boxes show mean, 25th, and 75th percentile for 25 experimental tanks. Whiskers are 5 and 95 percentiles of data.

Both the Ca and Cd concentrations in the experimental tanks remained essentially constant throughout the 72-h experiment. The variation through time (Fig. 1) was small and probably a result, primarily, of large scale dilutions required to reduce concentrations to a suitable analytical range. None of the slopes of the regressions between water concentration and time were significantly different from zero (*t*-test, $p = 0.05$, $n = 7$) for Ca, and all but one were not significantly different from zero for Cd.

There is almost no loss of Cd from marine clams during a depuration period of 12 d (Klummp and Burdon-Jones 1982) and in fresh water mussels over weeks of depuration (D. Maley

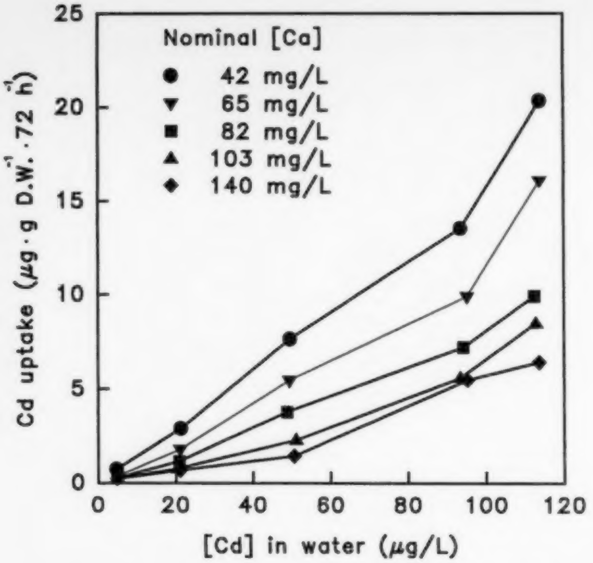

FIG. 2. Cd uptake by *E. complanata* as a function of total Cd in the experimental tanks, separated according to nominal Ca concentrations. Each point represents the average uptake for five individuals from that treatment.

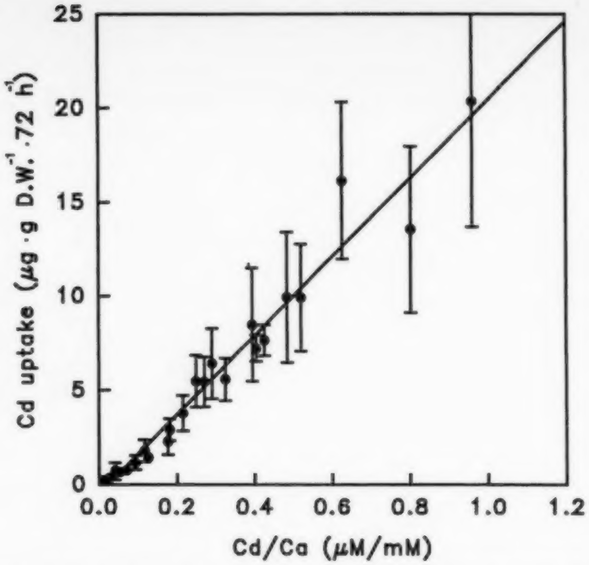

FIG. 3. Cd uptake by *E. complanata* as a function of the molar ratio of Cd to Ca. The equation for the least-squares regression line is given in the text.

Freshwater Institute, Winnipeg, personal communication). We conducted preliminary experiments to measure depuration (Wang 1990). Six mussels were placed in each of 25 different tanks containing a similar range of Cd:Ca ratios as those used in the uptake experiments. From each tank three mussels were measured directly after uptake while three were allowed to depurate for 5 d in clean water. No significant differences were found in activity levels between the two groups (*t*-test, $p < 0.0001$, $n = 25$). The procedure of Cd transfer from the soft tissue to the shell is very slow and temperature dependent (R.D. Evans, unpublished data). No detectable internal transfer of Cd to the shells occurred over the 72 h experimental period and our surface treatment of the exterior of the shell precluded external adsorption. Thus, it can be assumed that Cd removed from the water by the mussels was located in the soft tissues.

Uptake of Cd in each treatment group was calculated for the 72-h period from the determined activity levels and the known Cd specific activities. Uptake ranged from approximately 0.2 to 20 $\mu g \cdot g$ dry weight$^{-1} \cdot 72\ h^{-1}$ (Fig. 2). The Cd accumulation in the mussels' soft tissue, after 72 h of exposure, increased with an increase in the water Cd levels. At an approximately constant concentration of Ca, the Cd uptake is approximately linear with concentration of Cd in the water. This supports the theory that one important variable in Cd uptake is the concentration of free Cd^{2+} ions.

In Fig. 2, the data have been separated on the basis of nominal concentrations of Ca in the tanks. It is apparent that Ca concentrations in the water have a significant influence on Cd uptake by the mussels (confirmed by two-way analysis of variance) which can be observed at all Cd concentrations, and which can not be explained by differences in geochemical speciation. Cd uptake in soft tissues is positively correlated with the Cd concentration in the water but has a negative linear relationship with water Ca levels. Therefore the water Ca level depresses Cd uptake in mussel soft tissue. Similar results have been reported in field studies (O'Shea and Mancy 1978; Campbell 1987; Stephenson 1986). It is apparent from the analysis of these data that water concentrations of both Cd and Ca are needed to predict the Cd uptake in mussel soft tissue.

Given the significance of both variables, we related Cd uptake in mussels to the ratio of Cd to Ca in the water (Fig. 3). There is a very strong positive linear relationship defined by the equation:

$$(1)\quad \text{Cd uptake} \cdot \text{g dry wt}^{-1} \cdot 72\ \text{h}^{-1} = 0.029 \cdot \text{Cd/Ca} - 0.46$$

$$R^2 = 96.2\% \qquad p \leq 0.0001 \qquad n = 25.$$

Using data corrected for speciation (i.e., predicted free Cd^{2+} rather than total Cd) did not change the observed relationship. The less extensive experiment using six different treatments produced results with no significant difference from those of the original five-by-five experiment (non-parametric Mann–Whitney *U* test, $t = 1.4 < 1.96 = t_{0.05}$). Neither was there any significant difference between regression slopes ($t = 0.36 < 2 = t_{0.05}$), or regression intercepts ($t = 1.40 < 2 = t_{0.05}$) for the two experiments. The variation in Cd uptake within each treatment tank (vertical bars in Fig. 3) was mainly due to variation in the size of the mussels, with uptake rate inversely related to mussel dry weight.

Since the geochemistry of our systems was controlled and quite constant throughout the experiments, it is reasonable to assume that the major factor affecting Cd uptake is the Cd : Ca ratio. This observation supports the most popular theory of Ca regulation of Cd uptake in aquatic organisms — that Cd and Ca enter organisms through the same type of channel or binding sites on the cell membrane without preference. Because of this, both Cd and Ca in water are able to compete for these sites. Apparently, a higher ratio of Cd:Ca in water provides Cd with a greater possibility for adsorption onto the binding sites, which results in higher Cd accumulation in the tissue. These binding sites may exist for the uptake of group IIb metals in general. It would be illustrative to conduct similar tests with mixed-metal systems.

The significance of the ratio of Cd to Ca on Cd uptake in aquatic organisms has been noted in longer-term exposures in natural systems. For example, Stephenson (1986) observed the effect of Ca on the Cd uptake in a freshwater amphipod, *Hyalella azteca*, in some central Ontario lakes where the water Ca level ranged from 1.3 to 2.9 mg/L (soft water) and the Cd from 2 to 587 ng/L. In that study, the Cd accumulation in the amphipod had a strong positive linear relationship with the molar ratio of Cd to Ca ($\log(\mathrm{Cd/Dry\ Wt}) = 0.832 \log(\mathrm{Cd/Ca}) + 0.602$; $n = 51$, $R^2 = 74\%$, $p < 0.001$). Campbell (1987) studied *E. complanata* in 11 lakes in south-central Ontario with a range of water hardness including some located on the Trent water system, where the present study was carried out. Lake water concentrations of Ca and Cd ranged from 2.48 to 34.29 mg/L and 20 to 150 ng/L, respectively, and the ratio of Cd to Ca explained 86% of the variance in concentrations of Cd in the mussels.

Both of the above studies indicated that Cd accumulation in freshwater invertebrates has a strong relationship with the ratio of Cd to Ca in water column. However, both studies examined organisms from a range of lakes, making it hard to exclude the influence of other lake water variables (such as water DOC, $[O_2]$, temperature, pH and the interaction of other metals), and to separate geochemical and physiological processes. Our controlled laboratory experiment avoids the influence of most of these factors showing that the ratio of Cd to Ca in the water column is an important variable at the physiological level. Thus in lakes which are similar in all other factors, those with the highest Cd to Ca ratios will exhibit the highest Cd body burdens in its biota. Of course, true inter-lake variability will reflect the influences of geochemical differences as well. While this study was not designed to address the relative importance of geochemical versus physiological variables, it does stress the significance of the Cd to Ca ratio at the physiological level. It may be possible to separate the respective influences of each by comparison of our results with field studies. However, it is clear that any attempt to build a predictive model of Cd uptake in aquatic organisms with an uptake mechanism similar to *E. complanata* will require a component to reflect the importance of the Cd to Ca ratio.

The data also suggest an explanation for the relationship often found between Cd concentrations in organisms and lakewater pH (e.g., Campbell and Evans 1991). Cadmium and Ca tend to be inversely correlated in that soft, low pH waters will have relatively lower Ca concentrations and higher Cd concentrations than hard higher pH waters. For example, in Campbell's study this inverse relationship between Ca and Cd concentrations yields Cd:Ca ratios which vary between 0.002 and 15 μM Cd/mM Ca if all of the Cd and Ca were present in an available form. Thus the range of ratios found in nature may exceed that of our experiments. One interesting observation is that the biological potential for uptake of Cd would appear from our experiments to be much larger than that actually observed in natural populations. At a ratio of 1 μM/mM, uptake in our experiments was approximately 5 $\mu g\ \mathrm{Cd} \cdot g$ dry weight $^{-1} \cdot d^{-1}$. Such an uptake rate would quickly exceed even the highest concentrations found in similar systems (Campbell and Evans 1991). There are two possible explanations for this. First our experiments were conducted only in hard water systems. It is unlikely that the highest ratios which we used would be found ever in hard water systems except in very contaminated situations. Thus organism interaction with Cd may be different in soft water systems. More likely, however, is the possibility that geochemical factors control metal speciation such that only a small percentage of the total metal present in any aquatic system is in a biologically available form. This study indicates clearly the need for more work in which both the geochemical and physiological influences on Cd uptake by aquatic organisms is monitored.

Acknowledgements

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Fitting Surplus Production Models: Comparing Methods and Measuring Uncertainty

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Three approaches are commonly used to fit surplus production models to observed data: effort-averaging methods; process-error estimators; and observation-error estimators. We compare these approaches using real and simulated data sets, and conclude that they yield substantially different interpretations of productivity. Effort-averaging methods assume the stock is in equilibrium relative to the recent effort; this assumption is rarely satisfied and usually leads to overestimation of potential yield and optimum effort. Effort-averaging methods will almost always produce what appears to be "reasonable" estimates of maximum sustainable yield and optimum effort, and the r^2 statistic used to evaluate the goodness of fit can provide an unrealistic illusion of confidence about the parameter estimates obtained. Process-error estimators produce much less reliable estimates than observation-error estimators. The observation-error estimator provides the lowest estimates of maximum sustainable yield and optimum effort and is the least biased and the most precise (shown in Monte-Carlo trials). We suggest that observation-error estimators be used when fitting surplus production models, that effort-averaging methods be abandoned, and that process-error estimators should only be applied if simulation studies and practical experience suggest that they will be superior to observation-error estimators.

On emploie communément trois méthodes pour ajuster les modèles de production excédentaire aux résultats observés; il y a les méthodes de la moyenne d'effort, les estimateurs des erreurs de traitement ainsi que les estimateurs des erreurs d'observation. Nous comparons ces trois démarches au moyen d'ensembles de données réelles et simulées, et nous parvenons à la conclusion que ces méthodes conduisent à des interprétations largement différentes de la productivité. Les méthodes fondées sur les moyennes d'effort supposent que le stock est en équilibre relativement à l'effort récent; c'est rarement le cas, mais cela conduit ordinairement à une surestimation du rendement potentiel et de l'effort optimal. Ces méthodes produiront presque toujours ce qui semble être des estimations « raisonnables » du rendement soutenable maximal et de l'effort optimal, et la valeur statistique r^2 qui sert à évaluer la validité de l'ajustement peut donner l'illusion non fondée de confiance dans les estimations des paramètres qui sont obtenues. Les estimateurs d'erreurs de traitement donnent des estimations beaucoup moins fiables que les estimateurs des erreurs d'observation. Ces derniers conduisent aux estimations les plus faibles de rendement soutenable maximal et d'effort optimal; ce sont aussi les estimateurs les plus précis et qui comportent le moins d'erreur systématique (selon la méthode Monte-Carlo). Nous proposons d'utiliser des estimateurs d'erreurs d'observation lors de l'ajustement de modèles de production excédentaire, d'abandonner les méthodes de moyenne des efforts, et de n'appliquer les estimateurs d'erreurs de traitement que lorsque des études de simulation et que l'expérience immédiate donnent à penser que ces estimateurs seront supérieurs aux estimateurs d'erreurs d'observation.

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The simplest models of fish population dynamics consider only the changes in the exploitable biomass of the fishable stock. These models are generally called surplus production models (Ricker 1975) or more precisely biomass dynamics models (Hilborn and Walters 1992). In their simplest form, only a time series of catch and a relative abundance index (often standardized catch-per-unit effort, CPUE) are needed to estimate the model parameters. From these estimates, two quantities of considerable importance to management can be derived: the maximum sustainable yield (MSY) and the fishing effort at which MSY will be achieved (E_{MSY} — referred to as optimal effort, also sometimes denoted as f_{opt}). The major appeal of biomass dynamics models is that they can be applied in a situation in which the only data are catches and a relative abundance index. The computational simplicity of some of the fitting procedures for biomass dynamics models has meant that

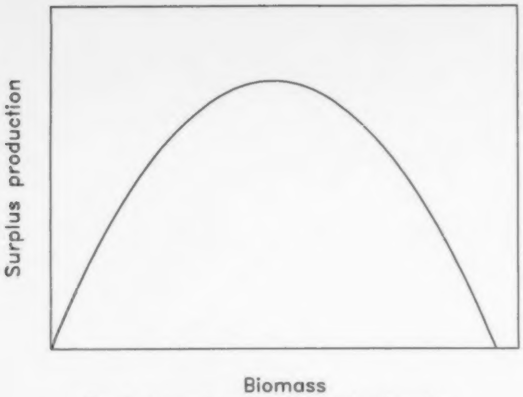

FIG. 1. A generic surplus-production function.

they have been widely used. Paucity of data is often a problem associated with a developing fishery, so that biomass dynamics models are frequently applied in this situation.

While age- and size-structured models (e.g., Deriso et al. 1985; Pope and Shepherd 1985; Gavaris 1988; Bergh and Johnston 1992) are used by many management agencies which have access to a time series of catch-at-age/size data, biomass dynamics models are still used in the management of many fisheries. This may be because the age/size compositions of the historic catches are not available or reliable. Another reason for considering biomass dynamics models is that in some situations they can provide more accurate and precise estimates of management-related quantities than more complex approaches (Ludwig and Walters 1985; Punt 1988, 1992a, b).

The method used to fit a biomass dynamics model to the observed data has been shown to be of much greater importance in terms of the reliability of estimated parameters than the algebraic form of the underlying population dynamics model (Punt 1988, 1992b). There are several ways to fit a biomass dynamics model to a set of observed data. However, only three have been widely used: (a) effort-averaging methods (Gulland 1961; Fox 1975), (b) process-error estimators (Walters and Hilborn 1976; Schnute 1977), and (c) observation-error estimators (Pella and Tomlinson 1969; Butterworth and Andrew 1984; Ludwig and Walters 1985)

Monte-Carlo simulation testing of estimation methods has shown that effort-averaging approaches are frequently highly (positively) biased, and process-error estimators are often very imprecise (Hilborn 1979; Uhler 1980; Punt 1988, 1992b). However, these two approaches continue to be used and even the most recent applications (e.g., Bartoo and Shiohama 1985; Sparre et al. 1989; Yeh et al. 1991) do not mention the existence of observation-error estimators nor do they appear to be aware of the deficiencies of the estimation methods being used. This is perhaps not surprising since the two most commonly-used textbooks in fisheries (Ricker 1975; Gulland 1983) do not mention process- or obervation-error estimators.

The objective of this paper is to review and compare the three methods and to show how the uncertainty associated with the estimates can be evaluated. No such comparison now exists; Hilborn and Walters (1993) provide a brief review of these methods but do not provide direct comparison nor do they show the computational procedures for calculating confidence bounds by likelihood profile. Butterworth and Andrew (1987) compare the methods by evaluating the precision of their estimates of $f_{0.1}$ harvesting strategy TACs for the four hake stocks off southern Africa, but they do not comment on the bias associated with these methods nor do they consider stock dynamics and perturbation histories other than those for hake.

The comparison includes two steps. First, the three estimation methods are applied to the actual data for three major marine resources [the Cape hake (*Merluccius capensis* and *M. paradoxus*) stock off northern Namibia, New Zealand rock lobster (*Jasus edwardsii*), and the south Atlantic albacore (*Thunnus alalunga*)]. These three data sets were selected because surplus-production models have been recently applied to them. Annual age-composition data do not exist for the rock lobster and albacore stocks so it is not possible to apply an age-based stock assessment procedure to them. Age-composition data for the years 1965 to 1989 are available for the hake stock, but are of questionable value. Punt and Butterworth (1989) applied a Laurec-Shepherd ad hoc tuned VPA to the data for this stock and found the relationship between fishing effort and fishing mortality to be extremely poor — to such an extent that the estimates of the quantities of importance to management were essentially worthless. The scientific basis for the TACs set by the International Commission for the South East Atlantic (ICSEAF) for this resource was thus based on fits of surplus-production models to catch and catch-rate data. The data sets for two of these three stocks (albacore and rock lobster) consist of a time series of increasing effort and decreasing index of abundance, which (Hilborn 1979) called a "one-way-trip" and showed were uninformative. The data set for the Cape hake stock is somewhat more informative as the index of abundance initially decreases, but then increases when effort is reduced.

The second part of the comparison involves a Monte-Carlo simulation evaluation of the estimation ability of the three methods. Simulations are based on each of the three stocks in order to detect the effects of the length of the data series and the extent of data contrast on estimation ability.

Models and Estimators

Biomass Dynamics Models

The essential feature of a biomass dynamics model-estimation procedure is the parameterization of the relationship between fishing intensity and long-term sustainable yield based on an assumed relationship between stock biomass and production. A number of alternative functional forms for this relationship exist, but all have the general shape shown in Fig. 1 [see the summary given in Punt (1988)]. Deterministically, all biomass dynamics models are of the form

$$(1) \quad B_{y+1} = B_y + g(B_y) - C_y$$

$$(2) \quad I_y = qB_y$$

where B_y is the (exploitable) biomass at the start of year y, $g(B)$ is surplus production as a function of biomass, for example either $g(B) = rB(1 - B/K)$ (Schaefer 1954 form), $g(B) = rB(1 - \log(B)/\log(K))$ (Fox 1970 form), or $g(B) = \frac{r}{p} \times B(1 - (B/K)^p)$ (Pella–Tomlinson 1969 form), r is the intrinsic growth rate parameter, K is the average biomass level prior to exploitation, q is the catchability coefficient, C_y is the catch during year y, and I_y is a index of relative abundance for year y.

Note that in the Pella–Tomlinson form, the *r/p* term is often omitted when the formula is presented. The parameter *p* in the Pella–Tomlinson form controls the asymmetry of the sustainable yield versus stock biomass relationship. It can be shown that the Schaefer form is equivalent to the Pella–Tomlinson form with $p = 1$ and that the Fox form is the limit of the Pella–Tomlinson form as $p \to 0$.

The various approaches to fitting biomass dynamics models to observed data considered in this paper (effort-averaging, observation-error, and process-error), differ in how error is introduced into Equations (1) and (2). The following discussion considers the case in which only one index of abundance is available.

Equilibrium Estimators

MSY and E_{MSY} can be estimated by assuming that the rate of change of biomass (i.e., dB/dt) is zero for all years (i.e., $B_{t+1} = B_t$ = Constant), and assuming that Equation (2) is exact. Now, it is often the case that the index of abundance $\{I_y\}$ is a time series of catch-rates (i.e., $qB_y = I_y = C_y / E_y$, where E_y is the fishing effort during year *y*). Solving Equation (1) for C_y after assuming that $B_{y+1} = B_y$, and after substituting $C_y / (qE_y) = B_y$, gives

$$(3)\quad C_y = \frac{rC_y}{pqE_y}\left(1 - \left(\frac{C_y}{qE_y K}\right)^p\right).$$

Equation (3) can then be solved for C_y / E_y:

$$(4)\quad C_y / E_y = \left((qK)^p - \frac{pq^{p+1} K^p E_y}{r}\right)^{1/p}.$$

If we define the first term $(qK)^p$ to be a new parameter *a* and the second term $\frac{pq^{p+1}K^p}{r}$ to be a new parameter *b*, then sum of squares estimates of the parameters *a*, *b*, and *p* can then be obtained by minimizing the quantity

$$(5)\quad \sum_y \left((C/E)_y - (C \hat{/} E)_y\right)^2$$

where $(C/E)_y$ is the observed catch-rate for year *y* and $(C \hat{/} E)_y$ is the model-predicted catch-rate for year *y*.

If the value of *p* is assumed to be one, estimates for the parameters *a* and *b* can be obtained using standard linear regression techniques. Estimates of MSY and E_{MSY} are obtained by means of the formulae

$$(6)\quad E_{MSY} = \frac{pa}{b(p+1)}$$

$$MSY = \frac{p}{b}\left(\frac{a}{p+1}\right)^{\frac{p+1}{p}}.$$

Effort-Averaging Approaches

It is recognized that fish stocks are rarely, if ever, in equilibrium. The most common approach to overcome this difficulty is to replace effort of the right hand side of Equation (4) by a weighted average fishing effort (Gulland 1961; Fox 1975). The common way in which to obtain the weighted average fishing effort for year *y* is by means of the formula suggested by Fox(1975), i.e.:

$$(7)\quad \bar{E}_y = \frac{k\,E_y + (k-1)\,E_{y-1} + \ldots + E_{y-k+1}}{k + (k-1) + \ldots + 1}$$

where *k* is the number of age classes being fished.

Combining Equation (4) with Equation (7) assumes recruitment is independent of spawner stock size; the stock size depends only on historical effort and not the size of the stock in the recent past (no spawner recruit effect). This assumption is not generally acknowledged by users of the method and effort-averaging methods are in practice an ad hoc approach for dealing with non-equilibrium conditions.

Process-Error Estimators

A process-error estimator is based on the assumption that the observations are made without error and that all of the error occurs in the change in population size [i.e., Equation (2) is assumed to be exact and Equation (1) is assumed to be subject to error]. For the Pella–Tomlinson form of the biomass dynamics function, substituting I_y for qB_y in Equation (1) and simplifying gives

$$(8)\quad I_{y+1} = I_y + \frac{r}{p} I_y (1 - (I_y / qK)^p) - qC_y.$$

For the case in which $p = 1$ (the Schaefer form), Equation (8) can be written in a form which is linear in its parameters:

$$(9)\quad I_{y+1} = (1+r)\, I_y - \frac{r}{qK} (I_y)^2 - qC_y.$$

In this form, estimates of *r*, *q*, and *K* can be obtained by multiple linear regression.

A large number of variants of this general approach exist. For example, Schnute (1977) provides the variant of Equation (9) which results from replacing Equation (1) with a continuous equation, while Lleonart et al. (1985) and Lleonart and Salat (1989) present a process-error estimator based on the concept of the inertia of a stock.

Observation-Error Estimators

An observation-error estimator (Pella and Tomlinson 1969; Butterworth and Andrew 1984; Ludwig and Walters 1985; Ludwig et al. 1988) is constructed by assuming that the population dynamics equation (Equation 1) is deterministic and that all of the error occurs in the relationship between stock biomass and the index of abundance. The stock biomass time series is estimated by projecting the biomass at the start of the catch series ($B_{initial}$) forward under the historic annual catches.

Assuming that the error in Equation (2) is multiplicative and log-normal with a constant coefficient of variation (i.e., $I_y = qB_y e^{\varepsilon}$, $\varepsilon \sim N(0; \sigma^2)$), the estimates of the model parameters ($B_{initial}$, *r*, *q*, and *K*) are obtained by maximizing the appropriate likelihood function:

$$(10)\quad L = \prod \exp\left|-\hat{v}_y^2 / (2\hat{\sigma}_v^2)\right| / (\sqrt{2\pi}\,\hat{\sigma}_v)$$

where the product is over all years (*y*) for which CPUE data are available:

$$\hat{v}_y = \log(C/E)_y - \log(C \hat{/} E)_y,$$

$$\hat{\sigma}_v^2 = \sum \hat{v}_y^2 / n$$

where n is the number of data points.

Equation (10) is an expression which is non-linear in its four parameters and so a non-linear minimization approach (e.g., Press et al. 1986) must be applied to obtain values for these parameters. It can be shown that the value of q which minimizes Equation (10) is given by the formula

$$(11)\quad \hat{q} = \exp\left\{\frac{1}{n}\sum_y \log(I_y/\hat{B}_y)\right\}.$$

Two other common assumptions regarding observation-error noise are that it is either additive and normal with a constant standard deviation (i.e., $I_y = qB_y + \varepsilon$, $\varepsilon \sim N(0; \sigma^2)$), or additive and normal with a constant coefficient of variation (i.e., $I_y = qB_y + \varepsilon$, $\varepsilon \sim N(0; (\sigma q B_y)^2)$).The maximum likelihood estimates of q for these choices of error structures are for an additive normal

$$(12)\quad \hat{q} = \sum_y I_y B_y \Big/ \sum_y B_y^2$$

and for a constant CV

$$(13)\quad \hat{q} = \frac{-\sum_y (I_y/B_y) + \sqrt{\left(\sum_y (I_y/B_y)\right)^2 + 4n\sigma^2 \sum_y (I_y/B_y)^2}}{2n\sigma^2}.$$

In practice, the likelihood surface is usually relatively flat near its maximum value (e.g., Rivard and Bledsoe 1978) and it is often necessary to introduce additional constraints (such as that $B_{\text{initial}} = K$). Fletcher (1978) discusses the source of this flatness while Punt (1990) found by simulation that for the Cape hake stock off northern Namibia, even in situations in which B_{initial}/K is substantially different from unity, better estimation performance is achieved by fixing B_{initial}/K at unity than by estimating it.

Uncertainty of Estimates and Confidence Bounds

One way to quantify the uncertainty associated with an estimate is to compute its confidence bounds. The three most frequently used methods are: asymptotic methods which assume that the likelihood function is quadratic near its minimum (Draper and Smith 1966), bootstrap approaches (e.g., Efron 1982; Punt and Butterworth 1993), and likelihood profile (Venzon and Moolgavkor 1988). Likelihood profile is used here because of its generality and computational ease.

The basic principles of likelihood and likelihood ratio can be used to define the confidence bounds for estimated parameters of any model for which the likelihood can be determined (Press et al. 1986, p. 532; Venzon and Moolgavkor 1988). For a single parameter p the confidence interval is defined as all values of parameter p that satisfy the inequality

$$(14)\quad 2[L(Y\,|\,p) - L(Y\,|\,p_{\text{best}})] \le \chi^2_{1,1-\alpha}$$

where $L(Y|p_{\text{best}})$ is the log likelihood of the most likely value of p and $\chi^2_{1,1-\alpha}$ is value of the chi-squared distribution with 1 df at confidence level $1-\alpha$.

Thus, the 95% confidence interval for p encompasses all values of p for which twice the difference between the log likelihood and the log likelihood of the best estimate of p is less than 3.84.

Likelihood profiles can be used to determine confidence bounds for the parameters either jointly or individually. The confidence region for n parameters is estimated based on the χ^2 distribution with n degrees of freedom. The likelihood profile method is preferred because it is computationally more efficient than bootstrapping and because many confidence regions are asymmetric (i.e., in two dimensions are often banana-shaped) rather than symmetric ellipses as assumed by asymptotic methods.

Application to Real Data

To ensure that comparisons made in this section are comparable, all data were fit to the Schaefer form of the surplus production function (i.e., $p = 1$), and for the effort-averaging method k (see Equation 7) was set to 3.

The results for each stock are given in the same format. The catch-rate data used in the assessments are presented in Table 1 and the estimates of a number of management-related quantities for each assessment method in Table 2. Note that the effort-averaging method does not provide estimates of biomass, so the estimates of quantities related to biomass are left out of Table 2. For each stock, a figure (Fig. 2, 4, and 6) is provided showing two plots. The first plot (panel a) shows the actual catches plotted against the corresponding efforts, with the equilibrium catch-effort curves obtained from each estimation method superimposed. The second plot (panel b) shows the catch-rate time series with the fits of the process- and observation-error estimators superimposed. Finally, for each stock, a figure (Fig. 3, 5, and 7) shows the point estimates of MSY and E_{MSY} and the 95% confidence bounds for these parameters computed using the method of likelihood profile.

South Atlantic Albacore

Up to 1990, the assessment of this stock by ICCAT (the International Commission for the Conservation of Atlantic Tuna) was based solely on the application of the effort-averaging method of Fox (1975). The value of p used in these assessments was 0.001 (Yeh et al. 1991).

The results of the three assessments are different in several respects, particularly regarding productivity (Table 2, Fig. 2 and 3). The effort-averaging method produces the most optimistic appraisal of the situation. According to this assessment, MSY is just larger than 28 000 t and the optimal effort is over 100 000 000 hooks. In only 6 yr is the annual catch larger than the estimated MSY (Table 1) which makes it rather difficult to see why the catch-rate should have dropped to roughly 30% of its initial size. The observation-error estimator is the least optimistic of the three assessments suggesting an MSY of only 19 650 t and an optical effort level roughly half that suggested by the effort-averaging method. The estimates of MSY and E_{MSY} obtained from the application of the process-error estimator are intermediate between the estimates obtained from the other methods (Table 2).

One of the reasons for the high estimates of MSY and E_{MSY} provided by the effort-averaging method is that there are a number of very high (E_{MSY}, MSY) data points (see Fig. 2a). Since the effort-averaging method assumes that the stock is relatively close to equilibrium, it attempts to pass the equilibrium catch-effort curve through these points. The other two methods assume instead that the catch taken reflects surplus production and reduction of standing stock. It is possible to infer from the trend in the catch-rate data that the stock has been declining over

TABLE 1. Catch and catch-rate data for the three stocks considered in this paper.

	New Zealand rock lobster		Northern Namibian hake		South Atlantic albacore	
Year	Catch (t)	CPUE	Catch ('000 t)	CPUE	Catch ('000 t)	CPUE (kg/100 hooks)
1945	809	3.49				
1946	854	3.38				
1947	919	3.18				
1948	1360	3.56				
1949	1872	1.79				
1950	2672	4.35				
1951	2834	2.33				
1952	3324	2.57				
1953	4160	2.88				
1954	5541	3.85				
1955	5909	4.16				
1956	6547	4.34				
1957	5049	3.70				
1958	4447	2.37				
1959	4018	2.46				
1960	3762	2.06				
1961	4042	2.21				
1962	4583	2.19				
1963	4554	2.44				
1964	4597	2.14				
1965	4984	2.18	93.510	1.78		
1966	5295	2.13	212.444	1.31		
1967	4782	1.86	195.032	0.91	15.9	61.89
1968	4975	1.53	382.712	0.96	25.7	78.98
1969	4786	1.32	320.430	0.88	28.5	55.59
1970	4699	1.45	402.467	0.90	23.7	44.61
1971	4478	1.40	365.557	0.87	25.0	56.89
1972	3495	1.09	606.084	0.72	33.3	38.27
1973	3784	1.23	377.642	0.57	28.2	33.84
1974	3643	1.12	318.836	0.45	19.7	36.13
1975	2987	0.92	309.374	0.42	17.5	41.95
1976	3311	1.02	389.020	0.42	19.3	36.63
1977	3237	1.00	276.901	0.49	21.6	36.33
1978	3418	1.05	254.251	0.43	23.1	38.82
1979	4050	1.09	170.006	0.40	22.5	34.32
1980	4190	1.03	97.181	0.45	22.5	37.64
1981	4058	1.01	90.523	0.55	23.6	34.01
1982	4331	0.98	176.532	0.53	29.1	32.16
1983	4385	0.88	216.181	0.58	14.4	26.88
1984	4911	0.85	228.672	0.64	13.2	36.61
1985	4856	0.84	212.177	0.66	28.4	30.07
1986	4657	0.81	231.179	0.65	34.6	30.75
1987	4500	0.84	136.942	0.61	37.5	23.36
1988	3128	0.68	212.000	0.63	25.9	22.36
1989	3318	0.62			25.3	21.91
1990	2770	0.54				

most of the period and so almost all of the catches reflect surplus production plus some fraction of the standing stock. This is why the equilibrium catch-effort curves for the process-error estimator and particularly the observation-error estimator lie below most of the observed data points (see Fig. 2a).

The estimates of current depletion obtained from the observation- and the process-error estimators are quite similar at roughly 32%. This result is not particularly surprising since the catch-rate series essentially determines the value of this quantity. However, there are major discrepancies between the estimates of current biomass provided by these two approaches. The observation-error estimator suggests that the stock was initially large with low productivity while the process-error estimator suggests exactly the opposite.

The apparent conflict between the process-error and observation-error point estimates of MSY and E_{MSY} are minor when the likelihood profile 95% confidence bounds are considered (Fig. 3). It is clear from this figure that the process-error estimator is extremely imprecise. Surprisingly, the results of the effort-averaging method appear to be quite precise and do not overlap with those of the (very precise) observation-error estimator. Hilborn (1979) showed that when the data are a one-way trip (constantly declining index of abundance, constantly increasing effort), a process-error estimator attempts

TABLE 2. Estimates of a number of management quantities obtained by applying three model-estimation procedures to the data in Table 1. Effort units are as in Table 1.

Quantity	Effort-averaging ($k = 3, p = 1$)	Observation-error estimator	Process-error estimator
		South Atlantic albacore	
r	—	0.328	0.620
q ($\times 10^4$)	—	26.71	43.72
K ('000 t)	—	239.6	153.4
MSY ('000 t)	28.09	19.65	23.78
B_{initial}/K	—	1.000	0.923
B_{current} ('000 t)	—	75.51	50.04
B_{current}/K	—	0.315	0.326
E_{MSY}	102.5	61.4	70.9
σ	—	0.111	0.736
		New Zealand rock lobster	
r	—	0.0659	0.448
q ($\times 10^5$)	—	2.461	6.184
K ('000 t)	—	129.0	52.6
MSY (t)	5278.5	2133.74	5897.7
B_{initial}/K	—	1.000	1.074
B_{current} (t)	—	21.15	0.87
B_{current}/K	—	0.163	0.166
E_{MSY}	2994.9	1337.9	3629.0
σ	—	0.207	0.568
		Northern Namibian hake	
r	—	0.379	0.304
q ($\times 10^4$)	—	4.360	2.701
K ('000 t)	—	2772.6	3448.2
MSY ('000 t)	365.8	263.2	262.3
B_{initial}/K	—	1.000	1.911
B_{current} ('000 t)	—	1646.3	2332.66
B_{current}/K	—	0.594	0.676
E_{MSY}	871.2	435.5	563.2
σ	—	0.124	0.662

to fit a 3-dimensional plane to a 2-dimensional line and will therefore be very imprecise.

Note from the formula given earlier that the observation-error estimation can use data from years where catch, but not catch-rate are known, and can use only occasional catch-rate data, while the process-error method can only use pairs of catch and catch-rate data.

New Zealand Rock Lobster

This stock (data from Breen 1991) has been assessed using both effort-averaging methods (Fox 1975) and process-error estimators. Qualitatively, the results for this stock (Table 2, Fig. 4 and 5) are the same as for South Atlantic albacore. The observation-error estimator provides the least optimistic appraisal once again, although this time it is the process-error estimator which is the most optimistic (if only slightly). Both the process-error and the observation-error estimators suggest that the stock is very highly depleted (to roughly 16–17% of K) although there are marked differences between the estimates of K and hence current biomass produced by these two estimators. The process-error estimator indicates a highly productive but small resource whereas the observation-error estimator suggests a highly unproductive resource which was initially quite large (Table 2).

Once again the results of the process-error estimator are very imprecise (Fig. 5). The extremely high precision associated with the effort-averaging method is due to the fact that the method is essentially performing a regression on the catch-rate versus effort data, which, being a one-way trip, are highly correlated. Users of effort averaging methods have often used this high correlation (r^2 is often >0.95) as an indication that the model is correct. Note that in this case all the catch-effort data points lie above the catch-effort curve estimated from the observation-effort estimator (Fig. 4a). This is because with the very low productivity rate estimated (0.0659, see Table 2), the catches are estimated to have been comprised almost entirely of standing stock. Surplus-production is estimated to have been almost nothing over the period considered.

Northern Namibian Hake

The Namibian hake data set shows more data contrast, since the catch-rate starts to increase towards the end of the time-series (Fig. 6). This results in the observation-error approach providing very precise estimates, while the process-error and the effort-averaging estimator are quite imprecise (Fig. 7). Based on the arguments of Hilborn (1979) discussed above, the process-error estimator is expected to be more precise for this series than it is for a one-way trip. While this appears to be true compared to the lobster data, it is hard to see that the hake estimates are

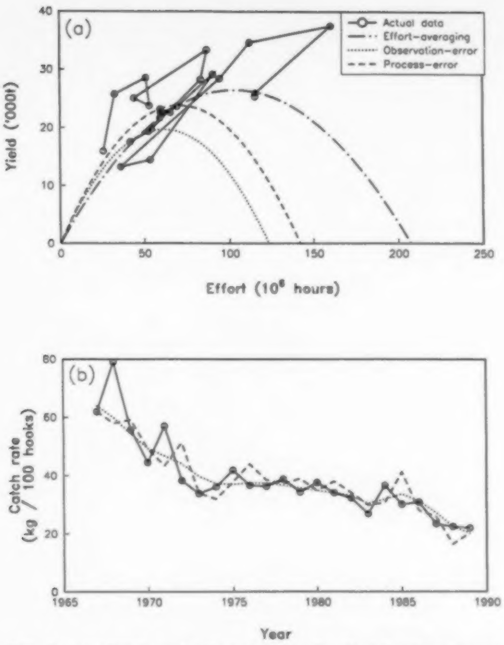

FIG. 2. (a) Catch and effort data for South Atlantic albacore with the equilibrium catch–effort curves obtained from the three estimation methods superimposed. (b) Catch-rate time series for South Atlantic albacore with the fits of the process-error and observation-error estimators superimposed.

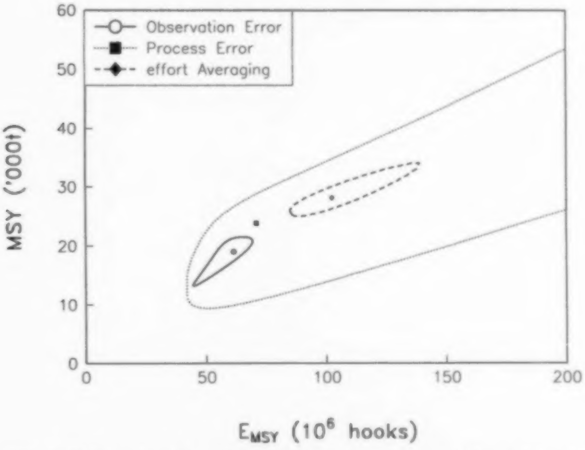

FIG. 3. Point estimates and 95% likelihood profile confidence bounds for the estimates of MSY and E_{MSY} obtained for South Atlantic albacore using the three alternative estimation methods.

more precise than the albacore estimates. The effort-averaging method has poorer precision because catch-rate is not correlated as closely with effort; the upturn in catch-rate reduces the correlation.

Qualitatively, the results for this data set are as before. The effort-averaging approach is much more optimistic than the other two approaches and the observation-error estimator is the most pessimistic. One disconcerting feature of the results for the process-error estimator is that it estimates that, at the start of exploitation (1965), the resource was almost twice its carrying capacity. While it is certainly possible that due to recruitment fluctuations, the biomass at the start of exploitation may have differed from the unexploited equilibrium biomass, it seems

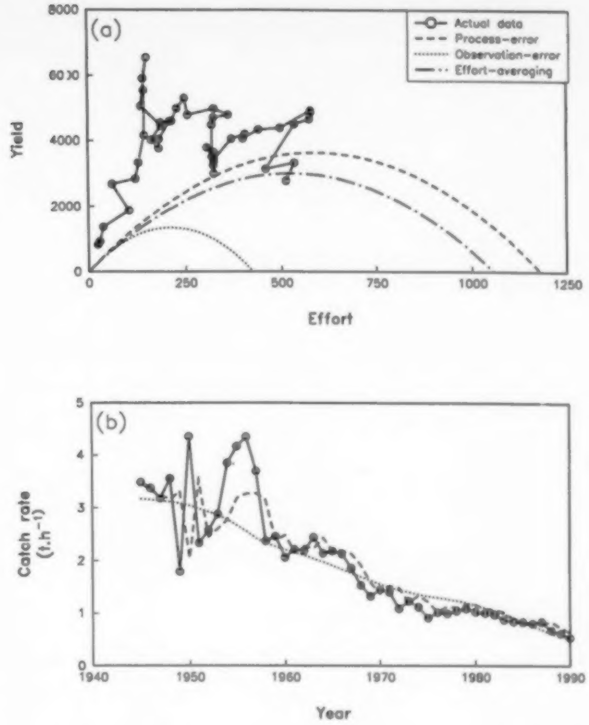

FIG. 4. (a) Catch and effort data for New Zealand rock lobster with the equilibrium catch–effort curves obtained from the three estimation methods superimposed. (b) Catch-rate time series for New Zealand rock lobster with the fits of the process-error and observation-error estimators superimposed.

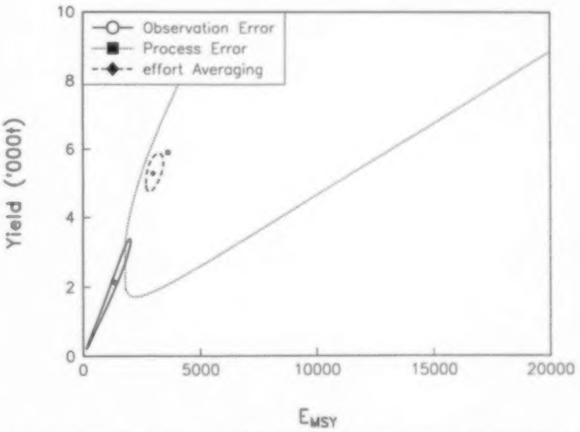

FIG. 5. Point estimates and 95% likelihood profile confidence bounds for the estimates of MSY and E_{MSY} obtained for New Zealand rock lobster using the three alternative estimation methods.

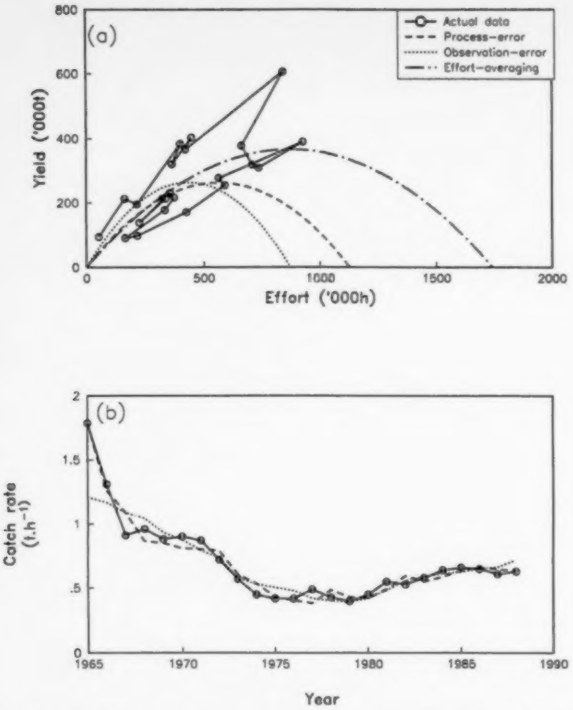

FIG. 6. (a) Catch and effort data for northern Namibian hake with the equilibrium catch-effort curves obtained from the three estimation methods superimposed. (b) Catch-rate time series for northern Namibian hake with the fits of the process-error and observation-error estimators superimposed.

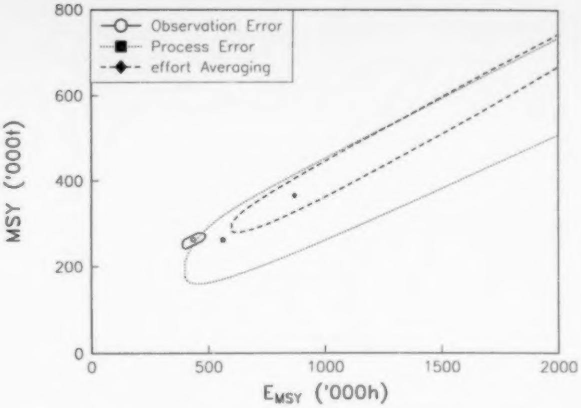

FIG. 7. Point estimates and 95% likelihood profile confidence bounds for the estimates of MSY and E_{MSY} obtained for northern Namibian hake using the three alternative estimation methods.

difficult to accept that the biomass could have been as different from *K* as is suggested by this assessment. The reason for this problem is, of course, the extremely high catch-rates for the initial years (see Fig. 6b). Whereas the observation-error estimator interprets this as observation-error, the process-error estimator assumes that the change in catch-rate between 1965 and 1967 reflects a real change (caused by a large process-error).

Summary of Results from Three Data Sets

It is not possible from the results of Table 2 and Fig. 2–7 to conclude which of the three estimators is best, because we do not know the true population parameters. However, certain general features can be concluded. First, the observation-error estimator provides the least optimistic appraisal while the effort-averaging method is the most optimistic. The process-error estimator is extremely imprecise. If the results of these assessments were to be pooled using, say, inverse variance weighting, the results for the process-error estimator would be given effectively no weight at all.

The extremely high precision associated with the observation-error estimator may be fallacious. The estimates of confidence bounds are naturally contingent on the model being correct. Punt and Butterworth (1993) using Monte-Carlo simulation, found that if the actual situation includes both process- and observation-error, estimates of variance obtained from the variance estimation procedures they considered were biased low by roughly 30%. Nevertheless, even if 30% is added to the confidence bounds, the results of observation-error estimator are still much tighter than those of the process-error estimator.

Monte-Carlo Testing

We were unable to conclude from the previous exercise which of the various methods was "best," primarily because it is unknown what the correct values for the management quantities of interest are. One way to overcome this problem is by testing the estimation performance of each method by means of Monte-Carlo simulation (Hilborn 1979; Uhler 1980; Ludwig and Walters 1985; Ludwig et al. 1988; Punt 1988).

Five hundred data sets were generated for each of the stocks based on the results of two of the estimation methods (process-error and observation-error estimators). It is not possible to generate data sets based on the results of the effort-averaging method because it does not estimate the biomass time-series. For the observation-error estimator, the data sets were generated by adding noise to the model-predicted catch-rate series using the equation

$$(15) \quad (C/E)_y^U = \hat{q}\hat{B}_y e^{\eta_y^U} \qquad \eta_y^U \sim N(0;\hat{\sigma}^2)$$

where $(C/E)_y^U$ is the catch-rate for year *y* in data set *U*, $\hat{q}$ is the estimate of *q* obtained by applying the observation-error estimator to the actual data, $\hat{B}_y$ is the estimate of biomass at the start of year *y* obtained by applying the observation-error estimator to the actual data, and $\hat{\sigma}$ is the standard deviation of the residuals of the fit of the observation-error estimator to the actual data.

For the process-error estimator, it would be desirable to apply the same basic procedure, namely:

$$(16) \quad I_{y+1}^U = I_y^U + \hat{r} I_y^U \left(1 - \frac{I_y^U}{\hat{q}\hat{K}}\right) - \hat{q} C_y + \eta_y^U$$

$$\eta_y^U \sim N(0;\hat{\sigma}^2)$$

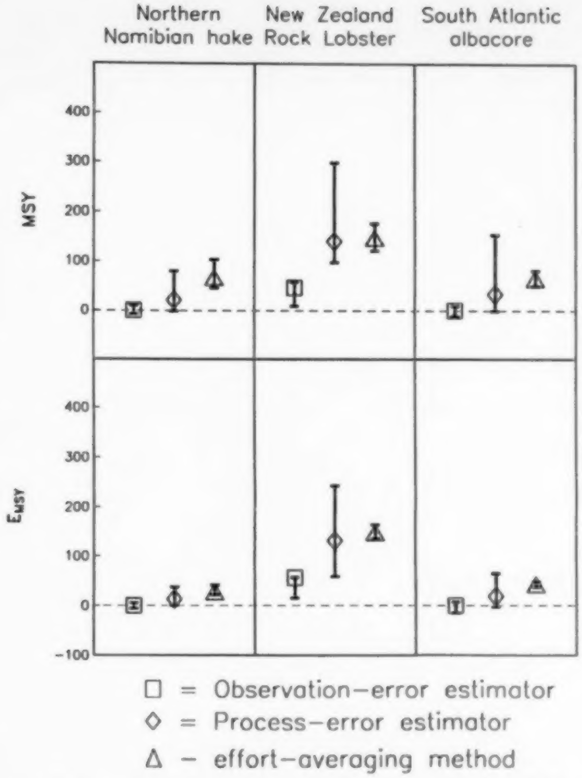

FIG. 8. Relative error distributions for MSY and E_{MSY} for the three estimation methods, for each of the three stocks, The data sets used in the calculation of this Figure were generated using the fit and assumptions of the observation-error estimator.

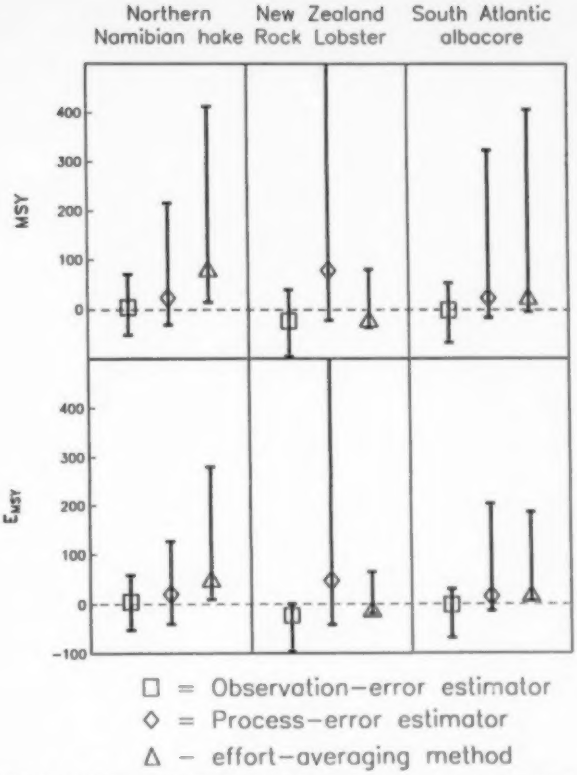

FIG. 9. Relative error distributions for MSY and E_{MSY} for the three estimation methods, for each of the three stocks. The data sets used in the calculation of this Figure were generated using the fit and assumptions of the process-error estimator. Only those data sets which corresponded to a population which was not extinct at the end of the catch time series were considered.

where $\hat{r}$, $\hat{q}$, and $\hat{K}$ are the estimates of the model parameters, and $\hat{\sigma}$ is the standard deviation of the residuals of the fit of the observation-error estimator to the actual data.

This prescription can result in population trajectories which are very different from those produced by the original assessment. This in principle, is not a problem. However, cases in which the population is rendered extinct before 1991 seem unrealistic. The obvious solution is to constrain the trajectories to those which are "similar" to the original data. This approach was used by Punt (1992b). Two cases have been considered, one in which all data sets that correspond to populations that did not go extinct before 1991 are considered, and one in which only those data sets that resulted in a current depletion which was within 10% of that reported in Table 2 are considered.

The results of this exercise (Fig. 8–10) are presented in the form of relative error distributions. The distributions are represented by their lower 5 percentiles, medians, and upper 5 percentiles.

For data sets based on the fits of the observation-error estimator (as described above), the results (Fig. 8) provide clear evidence that if the noise in Equations (1) and (2) is actually pure observation-error, then an observation-error estimator is to be preferred. The bias of the estimates provided by the observation-error estimator is only substantially larger than zero for the lobster example. The reasons for this are probably the fact that the estimate of "*r*" is very low for this stock (see Table 2) so that positive bias is more likely. The variance of the estimates of E_{MSY} and MSY are highest for the rock lobster example. This is a consequence of the higher value for σ used in this case (see Table 2).

As might have been anticipated, the effort-averaging approach is positively biased. This bias is lowest for Cape hake and largest for lobster. In all three cases, the estimates are fairly tight about the mean. This result suggests that it is not sufficient to perform analyses which suggest than an estimate is precise — it may nevertheless still be substantially biased. The process-error estimator is, in some respects, the poorest of the three approaches. It can be substantially positively biased (for rock lobster) and is the least precise of the three methods (as might have been expected from the results presented earlier).

The results for the process-error estimator suggest that when trajectories that go extinct are excluded (Fig. 9), we again see that the observation estimator is generally the least biased and most precise. The only exception is the lobster data set where the bias and precision of the effort-averaging method appear to be comparable to that of the observation-error estimator. In all cases the process-error estimator is both more biased and much less precise than the observation-error estimator.

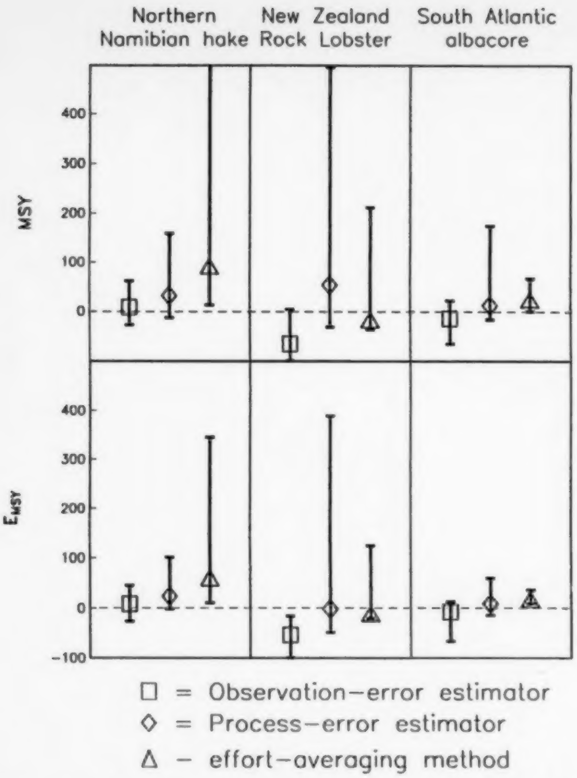

FIG. 10. Relative error distributions for MSY and E_{MSY} for the three estimation methods, for each of the three stocks. The data sets used in the calculation of this Figure were generated using the fit and assumptions of the process-error estimator. Only those data sets which corresponded to a current depletion which differed by less than 10% from the estimate provided by the process-error estimator were considered.

If we exclude all trajectories that are not similar to those for the real data (Fig. 10), the results do not change very much. The bias of the observation-error method for the rock lobster example is increased, however, and the process-error and effort-averaging methods are in fact less biased for the lobster example, although much less precise.

Discussion

The bias of the effort-averaging method, and the high variance of the process-error estimates make both of these methods extremely suspect. We believe that it is clear that the observation-error method should be expected to provide more precise and more accurate estimates of parameters, and if a single method is to be used, it should be the observation-error estimator. A rigorous assessment would include Monte-Carlo testing of alternative estimators as we have done in this paper. Such testing takes only a few hours on a desktop computer and should become standard practice.

Under no circumstances should agency staff, conference organizers, reviewers, managers or journal editors accept assessments or publications that are based on effort-averaging or process-error estimators only. The evidence presented in this paper, combined with previously published papers (Punt 1988; Punt 1992b; Hilborn and Walters 1992 etc.) make it clear that for most fisheries data sets observation-error estimators are superior.

Acknowledgments

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Biology of the Pacific Pomfret (*Brama japonica*) in the North Pacific Ocean

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Pearcy, W.G., J.P. Fisher, and M.M. Yoklavich. 1993. Biology of the Pacific pomfret (*Brama japonica*) in the North Pacific Ocean. Can. J. Fish. Aquat. Sci. 50: 2608–2625.

Abundances of Pacific pomfret (*Brama japonica*), an epipelagic fish of the North Pacific Ocean, were estimated from gillnet catches during the summers of 1978–1989. Two size modes were common: small pomfret <1 yr old, and large fish ages 1–6. Large and small fish moved northward as temperatures increased, but large fish migrated farther north, often into the cool, low-salinity waters of the Central Subarctic Pacific. Lengths of small fish were positively correlated with latitude and negatively correlated with summer surface temperature. Interannual variations in the latitude of catches correlated with surface temperatures. Large catches were made in the eastern Gulf of Alaska (51–55°N) but modes of small pomfret were absent here, and large fish were rare at these latitudes farther to the west. Pomfret grow rapidly during their first two years of life. They are pectoral fin swimmers that swim continuously. They prey largely on gonatid squids in the region of the Subarctic Current in the Gulf of Alaska during summer. No evidence was found for aggregations on a scale ≤1km. Differences in the incidence of tapeworm, spawning seasons, and size distributions suggest the possibility of discrete populations in the North Pacific Ocean.

L'abondance de la casthagnole mince (*Brama japonica*), un poisson épipélagique du Pacifique nord, a été estimée à partir des prises par filets maillants des étés 1978 à 1989. Deux classes de taille étaient communément trouvées : la petite casthagnole de moins de 1 an et la grande de 1 à 6 ans. Les uns comme les autres migrent vers le nord à mesure que la température s'élève, mais les plus gros se rendent plus loin au nord, souvent dans les eaux fraîches et peu salines de la partie centrale du Pacifique subarctique. Il existe une corrélation positive avec la latitude et une corrélation négative avec la température de l'eau de surface en été, et la longueur des petits poissons. Les variations d'une année à l'autre de la latitude des prises sont en corrélation avec la température de surface. Les grosses prises ont été faites dans la partie est du Golfe d'Alaska (51–55°N), mais les petits casthagnoles ne sont pas trouvés dans ce secteur, et les gros sont rares à la même latitude, mais plus à l'ouest. La casthagnole grossit rapidement durant ses deux premières années. Ce poisson se propulse avec ses nageoires pectorales et nage continuellement. Il se nourrit abondamment l'été des calmars gonatidés dans le Golfe d'Alaska ou dans la région où passe le courant subarctique. Rien n'indique qu'il y ait des agrégations de cette espèce sur une échelle égale ou inférieure à 1 km. Les différences dans le taux d'infestation par le ténia soulèvent la possibilité qu'il existe des populations distinctes dans le Pacifique nord.

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The Pacific pomfret, *Brama japonica* Hilgendorf (family Bramidae), is an abundant and widely distributed epipelagic fish in the North Pacific Ocean. It migrates from subtropical waters into feeding grounds north of the Subarctic Boundary during late spring and summer periods of high productivity (Neave and Hanavan 1960; Sasaki et al. 1981; Wada and Murata 1985; Brodeur 1988; Shimazaki 1989). During fall and winter, pomfret migrate rapidly into waters south of the Subarctic Boundary (Shimazaki and Nakamura 1981) where spawning occurs during winter through early spring near the Subtropical Frontal Zone in the western Pacific (Yoon and Shimazaki 1981; Shimazaki and Nakamura 1981).

The abundance of pomfret in the North Pacific is documented by their huge bycatch in the Japanese, Taiwanese, and Korean drift gillnet fisheries for flying squid (*Ommastrephes bartrami*). The estimated total number of Pacific pomfret caught by vessels of these nations was 1329 million and 82 million in 1990 and 1991, respectively (DiNardo and Kwok, unpublished data). In some years, the catch of pomfret was about one-half that of flying squid (Yatsu et al. 1993). Moreover, these bycatches may be underestimates because pomfret drop out of the gill nets during retrieval and accurate counts are not possible when large numbers are caught (Int. North Pac. Fish. Comm. 1991). LeBrasseur et al. (1987) estimated that 100–300 million pomfret may have been discarded annually in the Japanese fishery alone. Drift gill nets fished for flying squid off British Columbia also caught large numbers of pomfret; sometimes it was the most abundant species (Robinson and Jamieson 1984; Jamieson and Heritage 1987).

Pacific pomfret is greatly underutilized as a food fish. Some are landed in countries bordering the western Pacific, and a few are marketed in United States. It has excellent flavor to the palate

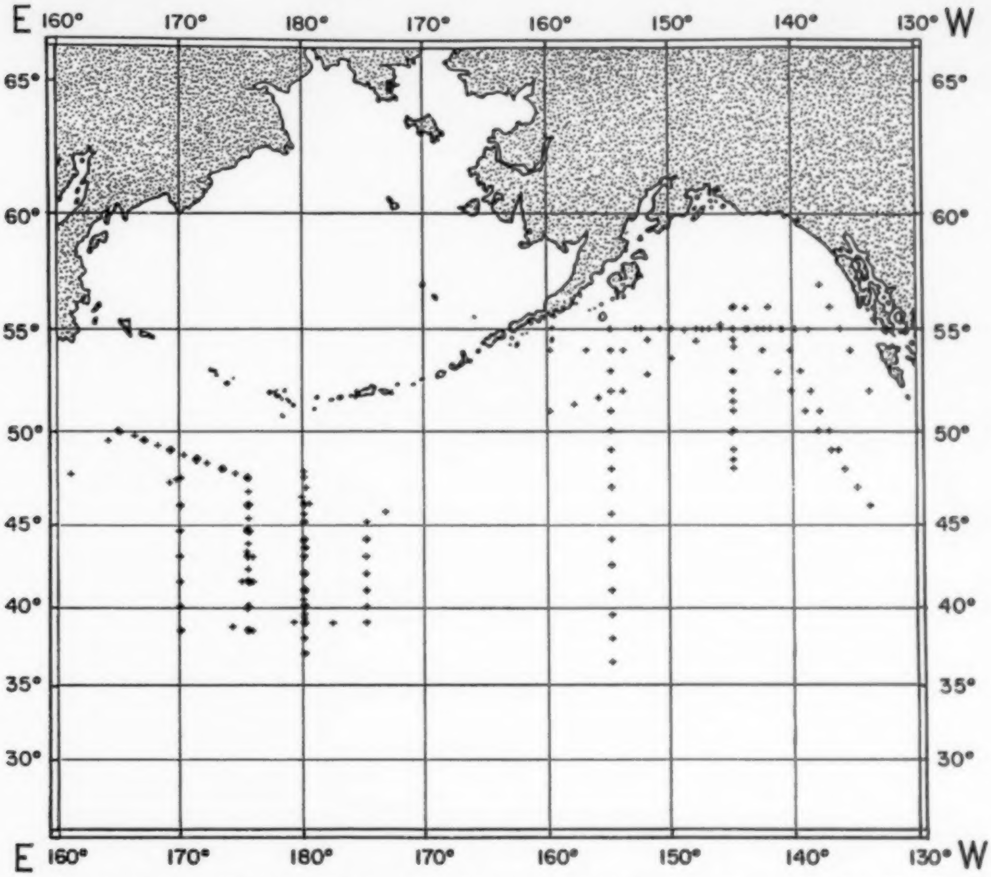

FIG. 1. Location of gillnet stations, 1978–89, by training ships from Hokkaido University. The transects along 180°, 175°E, and 170°E were repeated during most years (see text).

of North Americans (Elwell 1987).

Because of the abundance of Pacific pomfret, its important ecological role in these oceanic regions, and its potential for sustaining a large commercial fishery, better understanding of the biology and ecology of this species is needed (Shimazaki 1989). Based on results of 12 yr of gillnet cruises by Hokkaido University, we present information on geographic variations in size distributions, interannual trends in abundance relative to ocean conditions, patchiness of catches in gill nets, age and growth, food habits and parasites, and observations on swimming behavior of Pacific pomfret.

Methods

Data on catches of Pacific pomfret in gill nets together with oceanographic data were collected during summer cruises from 1978 to 1989, by the Hokkaido University Training Ships (T/S) Oshoro Maru along 180° and in the Gulf of Alaska (130°–165°W) and Hokusei Maru between 165°E and 180° (Fig. 1). Gill nets, 4550–6600 m long and 6.7 m deep, were fished overnight. Gill nets fished from the Oshoro Maru usually included 150–250 m (3–5 tans, one tan = 50 m) each of 29, 33, 37, 42, 48, 55, 63, 72, 82, 93, 106, 138, 157, 179, and 204 mm (stretched) mesh, 1000–1400 m (20–28 tans) each of 115 and 121 mm mesh, and 500–1000 m (10–20 tans) of 130 mm mesh. Gill nets fished from the Hokusei Maru usually included 50–150 m (1–3 tans) of 19, 22, 25, 29, 33, 42, 48, 55, 63, 72, 82, 93, 106, 138, 157, 179, 204, and 233 mm mesh, and 500–1000 m (10–20 tans) of 112, 115, 118, and 121 mm mesh (see Hokkaido University 1979–90 for details on gill nets, catches and oceanographic observations). Of the 385 sets that were completed during these years, 253 caught 92 439 pomfret. A total of 20 799 was measured to the nearest millimeter fork length (FL), including samples from each mesh size.

Temperature and salinity were measured at each gillnet station, and usually midway between gillnet stations, from the surface to a depth of at least 700 m. Dynamic height anomalies relative to 700 dbar were calculated by integrating specific volume anomalies (Fofonoff and Millard 1983) over pressure.

Estimating Abundance of Pacific Pomfret

A gill net fishing equal numbers of tans in a geometric size series of meshes, with each mesh approximately 14% larger than the preceeding one, is non-size-selective for Pacific pomfret from 160 to 520 mm FL (Shimazaki et al. 1981). The non-selective gill net described by Shimazaki et al. (1981) consisted

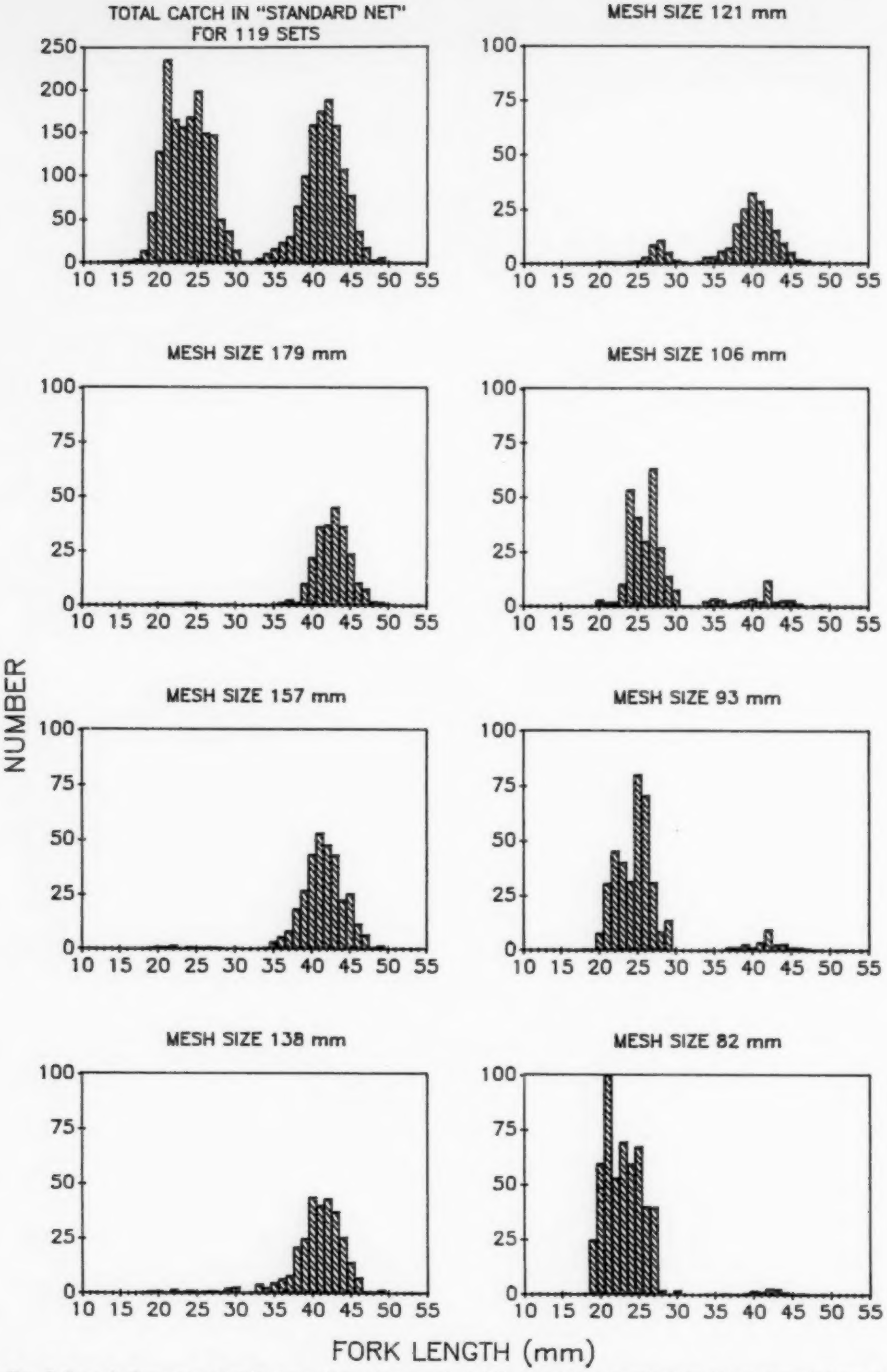

FIG. 2. Length–frequency distributions of pomfret caught in 119 sets of the "standard net" for the mesh sizes of Shimazaki et al's. (1981) non-selective gill net (excluding the 204 and 233 mm meshes). (*Fig. 2 concluded next page*)

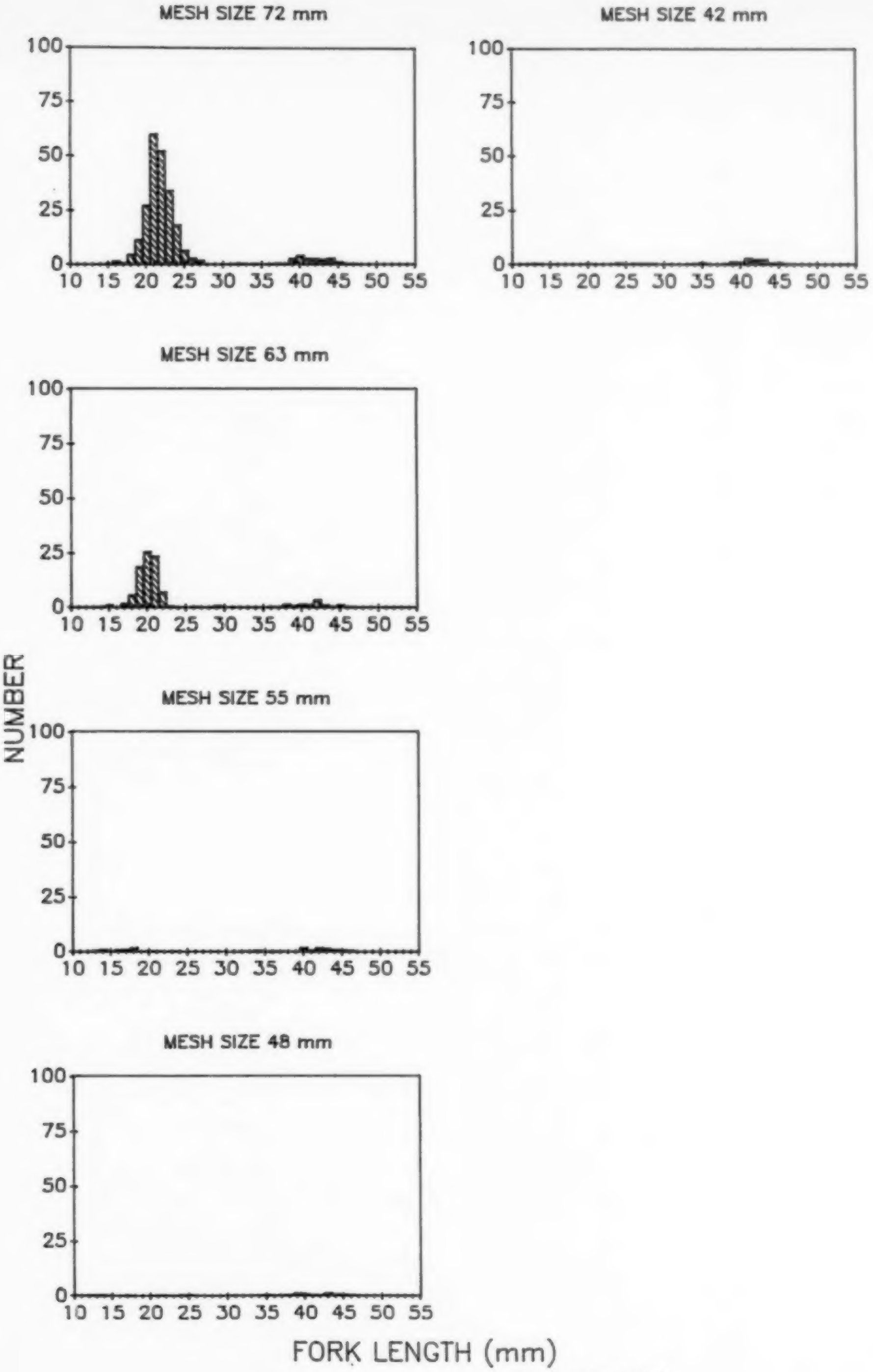

FIG. 2. (*Concluded*)

of 42, 48, 55, 63, 72, 82, 93, 106, 121, 138, 157, 179, 204, and 233 mm meshes. Standardized catches for each mesh size of this geometric series through 179 mm mesh (Fig. 2) show that small meshes (<106 mm) caught predominantly small (≤330 mm FL) pomfret and large meshes (121–179 mm) caught mainly large (>330 mm FL) pomfret. In our study, gill nets departed from the "research net" described by Shimazaki et al. (1981) chiefly in that many tans of 112, 115, 118, 121, and 130 mm mesh were

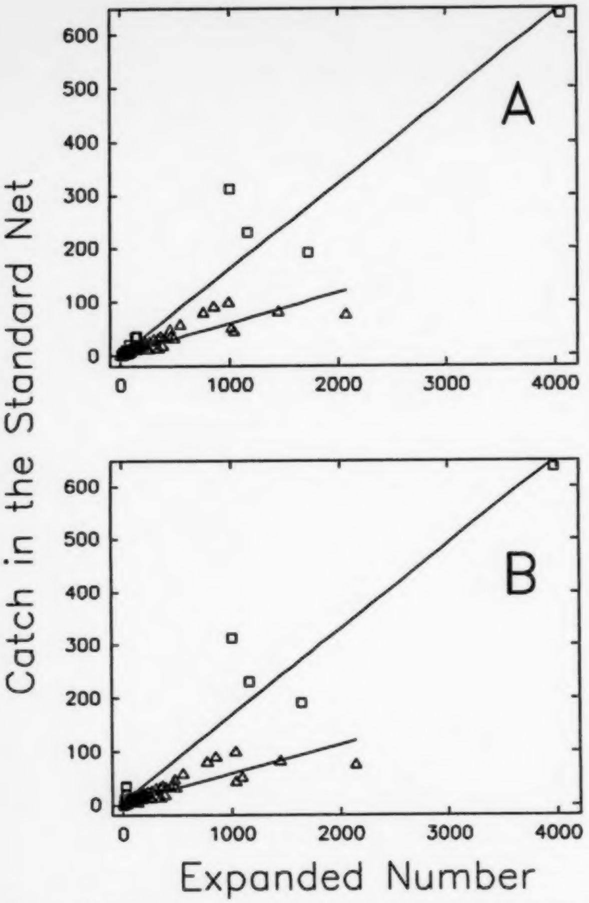

FIG. 3. (A) Regressions of catch of large and small pomfret in the standard net on the numbers of measured small and large pomfret expanded to equal the total catch in the set. Data are from 157 sets for which catch in the standard net could be determined directly (Method 1 in text). These regressions were used to estimate catch in the standard net in 69 sets where data were inadequate to determine catch directly, but where fish were measured from most mesh sizes (Method 2 in text). Small fish (squares), $n = 78$, $Y = 0.16X + 1.47$, $r = 0.97$. Large fish (triangles), $n = 137$, $Y = 0.06X + 3.41$, $r = 0.89$; (B) Same as 3(A), but expansions were based on the numbers of large and small fish in the 121 mm mesh only. Regression between catch in the standard net and the expanded numbers of small and large pomfret from 121 mm mesh were used to estimate catch in 22 sets where only fish from the 121 mm mesh were measured (Method 3 in text). Small fish (squares), $n = 29$, $Y = 0.16X + 5.69$, $r = 0.97$. Large fish (triangles), $n = 121$, $Y = 0.05X + 3.9$, $r = 0.88$.

fished in addition to the standard geometric series. Often these non-standard meshes accounted for over 50% of all tans in the gill net. In addition, small meshes between 19 and 37 mm were fished. Gillnet configurations consisted of as few as 12 and as many as 23 different sizes of mesh, and the total numbers of tans fished ranged from 63 to 133.

Because of the variability in net configuration, and the consequent variability in selectivity for different sizes of pomfret, raw catches in the different gill net sets were poor estimates of size-specific abundances of pomfret. To estimate relative abundance of small (≤330 mm FL) and large (>330 mm FL) pomfret caught in different sets and cruises, we converted raw catch in each gill net set to an estimated catch in the standard net described by Shimazaki et al. (1981). Depending on the completeness of catch data, one of three methods was used to make this conversion:

Method 1

Complete mesh-by-mesh catch data for pomfret were available for 157 sets. Total catches and size-frequency distributions of fish from subsamples were known for each mesh size in these gillnet sets. Total numbers of small and large fish caught in each mesh size were estimated by expanding the numbers in the measured subsample for each mesh. Catch data for each mesh were converted to catch/tan by dividing by the number of tans fished of the corresponding mesh. Catches/tan in each mesh in the geometric series described by Shimazaki et al. (1981), with the exception of the 204 and 233 mm meshes that were often missing, were added to give catch of large and small fish in a standard non-selective net in which one tan of each standard mesh was fished. This standardized catch, called "catch in the standard net," was the basis for our comparisons of abundance.

Method 2

In 69 gillnet sets, total catch of pomfret in the gill net and the size-frequency distributions of subsamples from the different meshes were known, but catch in each mesh was not known. Therefore, no estimate could be made of total catch of large and small fish in each mesh. Because catch in the standard net could not be calculated directly, it was estimated in two steps. The ratio of large and small fish in the measured sample from all meshes combined was expanded to the entire catch:

$$\text{No. small (large) fish} = \frac{\text{measured small (large) fish}}{\text{all measured fish}} \times \text{total catch.}$$

The expanded numbers of large and small fish were converted to catches in the standard net using regressions of these two abundance estimates for large and small fish from the 157 gillnet sets where catch in the standard net could be calculated directly (Method 1) (Fig. 3A). Because of the many extra meshes in the size range from 112 to 130 mm, the abundance of large fish relative to small fish in the measured sample was usually greater than that in the standard net. High selectivity of the gill nets for large fish and low selectivity for small fish are indicated by the lower slope of the regression for large fish than for small fish (Fig. 3A).

Method 3

In 22 sets, fish were measured in the 121-mm mesh and, at most, in one additional mesh. Catches in the standard net were converted from numbers of large and small fish in the 121-mm mesh expanded to equal the total catch in the set using regressions for large and small fish from the 157 complete sets, as in Method 2. The regressions are similar to those of Method 2 (Fig. 3B), indicating that the 121-mm mesh and the non-standard gill net caught similar ratios of large and small fish (see Fig. 2). In five sets, pomfret were caught, but none was measured and abundance was not estimated.

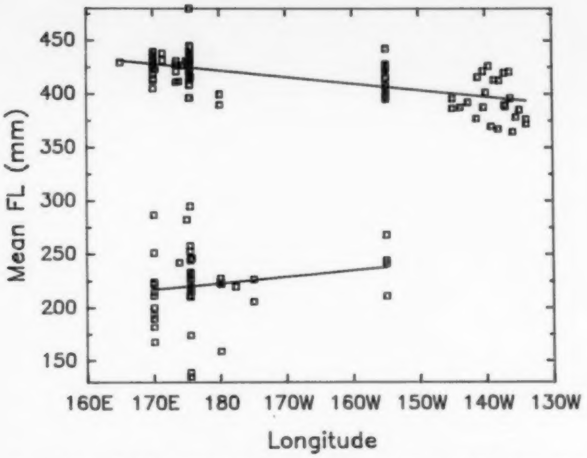

FIG. 4. Mean fork lengths of the distinct modes of small and large pomfret caught in the standard net during July vs. longitude of capture based on 157 sets with complete catch by mesh data.

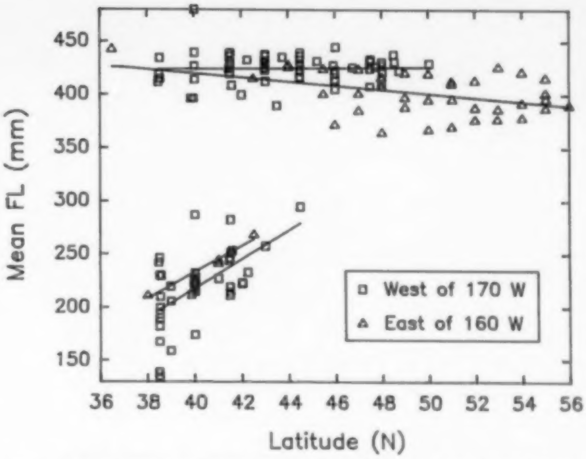

FIG. 5. Mean fork lengths of the distinct modes of small and large pomfret during July vs. latitude (squares, west of 170°W; triangles, east of 160°W) based on 157 sets with complete catch by mesh data.

Aggregation of Catches

The patchiness in abundance of pomfret caught within gill nets was examined from numbers of fish caught in contiguous tans of the same mesh size. In 26 instances, 10–20 tans of either 115, 121, or 130 mm mesh were fished in a continuous series. Coefficients of dispersion (variance/mean ratios) of the catch per tan were tested against a random distribution, assuming that they were distributed as a chi-square variate.

Age and Growth

Sagittal otoliths were removed from Pacific pomfret collected in the eastern North Pacific (135°–155°W) onboard the Oshoro Maru during the summers of 1985 and 1987, and stored dry (1985) or in a 50% glycerin-water solution with thymol added as a preservative (1987). A few small, whole pomfret were frozen in 1985 and otoliths were removed and fish lengths and sex recorded in the laboratory. All otoliths were cleaned in the laboratory and stored in 80% ethanol.

Otoliths were submerged in water on a black background, and distal surfaces were examined at 32–80× magnification under a Wild M3 dissecting microscope with reflected light. A white or opaque band, representing rapid growth, and an adjacent narrower dark or translucent band, deposited during periods of slow growth, were interpreted as 1 yr of growth, although increment periodicity remains unvalidated for this epipelagic species. The type of growth on the edge of the otolith was noted. Transverse and longitudinal sections were taken from many otoliths.

Narrow, fine-scale bands, presumed to represent daily growth increments, were counted on sagittal sections of otoliths from the smallest pomfret following the methodology of Boehlert and Yoklavich (1985). Increments were counted at 1000× magnification using a compound microscope and transmitted light. Increments appeared as distinct, concentric rings around the focus but became progressively smaller and less distinct toward the edge of the otolith.

Food Habits

Major prey taxa were determined from examination of stomach contents of 979 Pacific pomfret caught east of 180°. In 1980, stomachs were removed, frozen at sea, and preserved in 10% formalin ashore. Food items were identified and major prey taxa were weighed. In 1981, frequency of occurrence of major prey was calculated. In 1984, 1985, 1986, and 1987, percent composition of major prey taxa was estimated visually at sea. In all years, common prey taxa were preserved and identified. Indices of stomach fullness were estimated as 0 (empty), 1 (to $\frac{1}{4}$ full), 2 ($\frac{1}{4}$ to $\frac{1}{2}$ full), 3 ($\frac{1}{2}$ to $\frac{3}{4}$ full), and 4 (full, rugae of stomach not apparent, stomach lining thin). State of digestion of stomach contents was also noted as fresh (1), partially digested (2), and well-digested (3).

Parasites

Pacific pomfret from stations east and west of 180° were examined for larval tapeworms imbedded in the flesh. In all, 53 fish were frozen and examined in the laboratory ashore. Each fish was filleted and each of the four fillets from a fish, with skin removed, was candled with a lamp to detect tapeworms. When possible, the entire parasite was removed for identification.

Swimming Behavior

In 1987, pomfret caught on hook-and-line from a depth of about 20–30 m during the daytime were immediately placed in a 90 × 122 × 75 cm deep tank with circulating sea water on board the Oshoro Maru. Swimming behavior and gill ventilation were observed over 24 h.

Results and Discussion

I. Geographic Variations in Size Distribution

The size distribution of the catches was almost always unimodal or bimodal with clearly separated modes (see also Machidori and Nakamura 1971; Wada and Murata 1985; Shimazaki 1989), enabling classification of fish into two size groups: small fish (130–330 mm) and large fish (331–500 mm) (Fig. 2). Few fish of intermediate size were captured. Only 26 of over 20 000 pomfret measured were over 500 mm FL, and only three were over 600 mm FL. The largest was 614 mm FL.

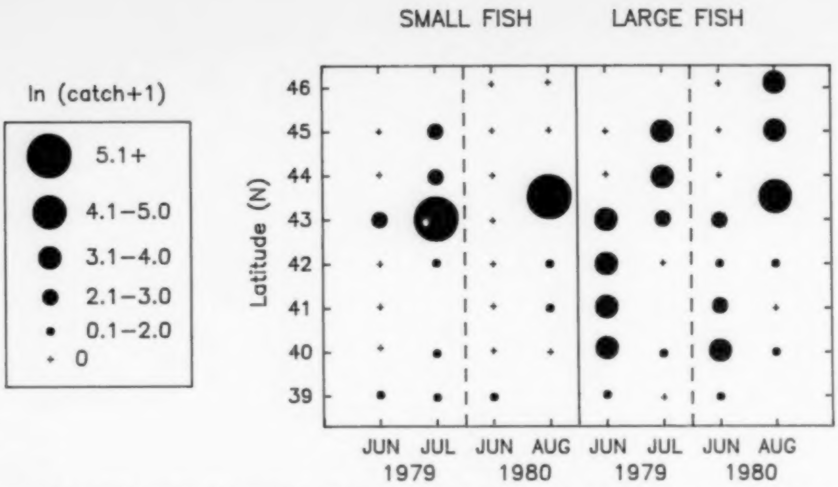

FIG. 6. Catches of small (≤330 mm FL) and large (>330 mm FL) pomfret in the standard net during early and late transects along 180°.

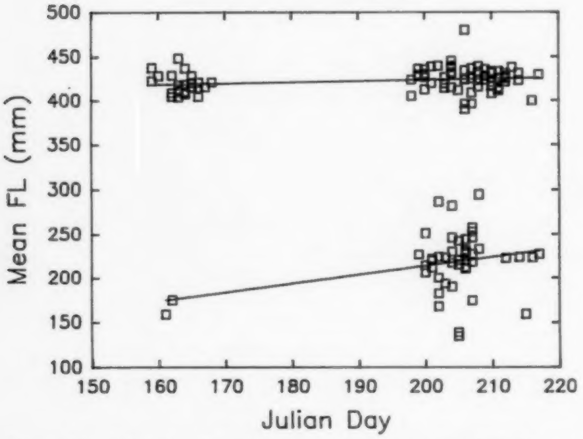

FIG. 7. Mean fork lengths of the distinct modes of small and large pomfret in the standard net vs. Julian date of capture for sets west of 170°W based on 117 sets with complete catch by mesh data.

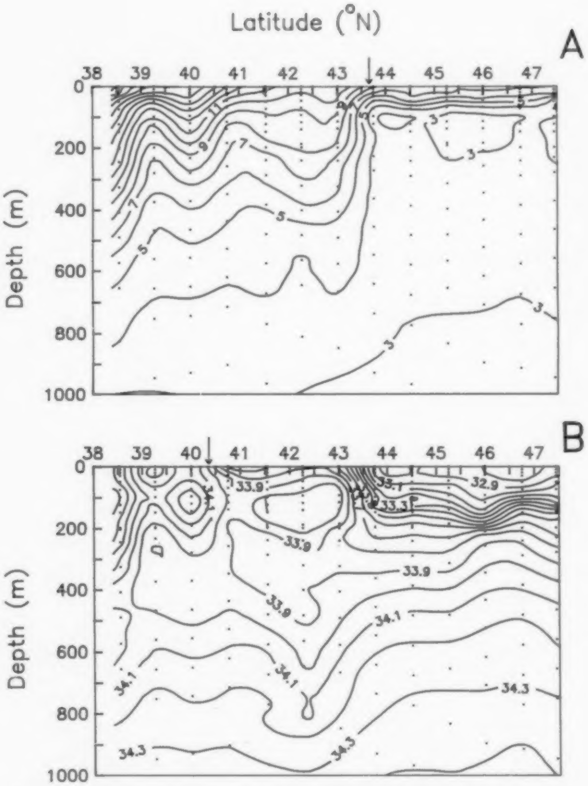

FIG. 8. Vertical section of (A) temperature and (B) salinity along 170°E during July 1983. The Subarctic Boundary is indicated by the intersection of the 34 ‰ isohaline with the surface, and the boundary between the Transitional and the Central Subarctic Domains of subarctic water is shown by the rapid descent of the 4°C isotherm below 100 m.

The frequency of bimodal catches and the mean length of pomfret varied by location of capture. During July, when most sets were made, catches of small fish and bimodal catches of small and large fish were common in the western North Pacific (west of 170°W), whereas only four sets included modes of small pomfret in the Gulf of Alaska (Fig. 4). The mean lengths of the modes of large pomfret, but not small pomfret, decreased from the western Pacific to the Gulf of Alaska during July ($r = -0.65$, $P < 0.01$, $n = 81$, Fig. 4).

The size of pomfret in the standard net also varied with latitude. Modes of both small and large fish were common south of 44°N during July, but only large fish were caught north of 44°N. (Fig. 5). The largest percentage of sets with only modes of small pomfret occurred in southern waters, between 39° and 40°N. In addition, the mean FL of small fish was positively correlated with latitude ($P < 0.01$) both east of 160°W and west of 170°W, indicating that the smallest pomfret inhabited more southerly waters during the summer and did not migrate in high numbers into waters north of 44°N in this region. This trend was not found for large pomfret during July. Mean size of large pomfret decreased ($P < 0.05$) with latitude east of 160°W, but no

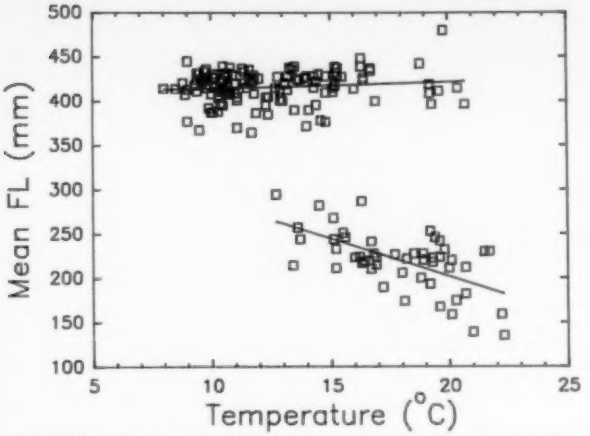

FIG. 9. Mean fork lengths of the distinct modes of small and large pomfret in the standard net vs. sea surface temperature based on 157 sets with complete catch by mesh data.

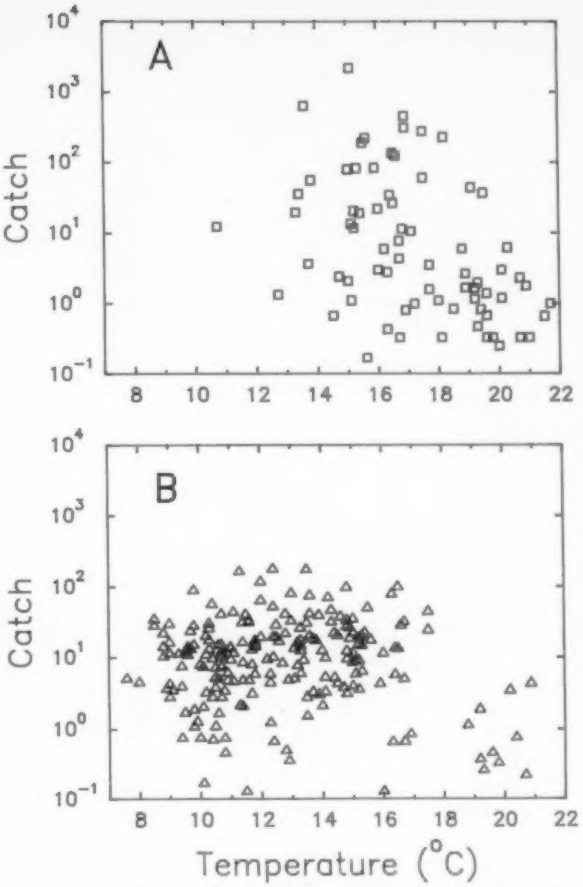

FIG. 10. Numbers of pomfret caught in the standard net vs. sea surface temperature for distinct modes of (A) small pomfret and (B) large pomfret.

significant trend with latitude was found for large pomfret west of 170°W.

Mean length of pomfret increases from south to north during the summer in subarctic waters (Sasaki et al. 1981). Shimazaki (1989) reported that during the winter pomfret were only caught south of the Subarctic Boundary, whereas in early summer small pomfret (< 310 mm FL) were distributed south of the Subarctic Boundary (38°30′ to 43°N) while large pomfret were found in waters north of the boundary (45°30′ to 47°30′N, along 175°30′E). These trends indicate the more northerly migration of large fish during the summer.

North-south transects along 180°, conducted during June, July, or August, 1979 and 1980, clearly illustrate the northward movements of both small and large pomfret (Fig. 6). During these years, small pomfret (all fish ≤330 mm FL even if not in a distinct mode) were caught 2° farther to the north in July than in June 1979, and 4.5° farther to the north in August than in June 1980. These changes corresponded to movements northward of 2.7 and 5.1 miles per day, respectively. Large pomfret moved north by 2° in 1979 and 3.1° in 1980, corresponding to 2.7 and 3.8 miles per day, respectively.

Mean size of both small and large pomfret increased with date of capture ($P = 0.05$ and 0.07 respectively), although only two samples of small fish were measured in June (Fig. 7). The increase in size of small and large pomfret was about 1.0 mm and 0.15 mm/d, respectively. These rates are about twice the growth rates of age 0+ and 1+ pomfret estimated from otoliths (see Age and Growth section), and possibly result from size-selective migration of fish.

II. Pomfret Distribution in Relation to Oceanography

Pacific pomfret are associated with the Transitional Domain of the Subarctic Water Mass during the summer months (Shimazaki and Nakamura 1981; Shimazaki 1989; Pearcy 1991). This region is located north of the prominent Subarctic Boundary (often defined by the vertical ascent or the surface intersection of the 34 ‰ isohaline) and south of the boundary between the Transitional and Central Subarctic Domains (where the 4°C isotherm plunges below 100 m) (Dodimead et al. 1963; Favorite et al. 1976). These two boundaries often coincide with strong latitudinal gradients of both temperature and salinity (Fig. 8), and with regions of relatively strong gradients in dynamic height and hence strong geostrophic currents (see Fig. 11E, 12E).

Catches vs. sea surface temperature

The mean lengths of pomfret varied with sea surface temperatures (Fig. 9). The mean size of small fish decreased with increasing temperature ($r = -0.62$; $P < 0.01$), as expected if migration to the north is size-dependent. No trend in mean size with temperature was apparent for the large fish ($r = 0.12$; $P > 0.05$).

Small pomfret were rarely caught in waters where the sea-surface temperature was less than 13°C, whereas large fish were commonly found where temperatures were from 8 to 13°C (Fig. 10). Thus the 13°C isotherm may be a boundary to the northward migration of small fish. During June and July, small pomfret inhabited waters over 4°C warmer than large pomfret (mean 17.3°C vs. 12.8°C).

Wada and Murata (1985) also reported size segregation of pomfret by temperature with large fish most numerous at surface temperatures of 13–17°C, while small fish were mainly found in 17–21°C water. Large fish were usually more common in waters

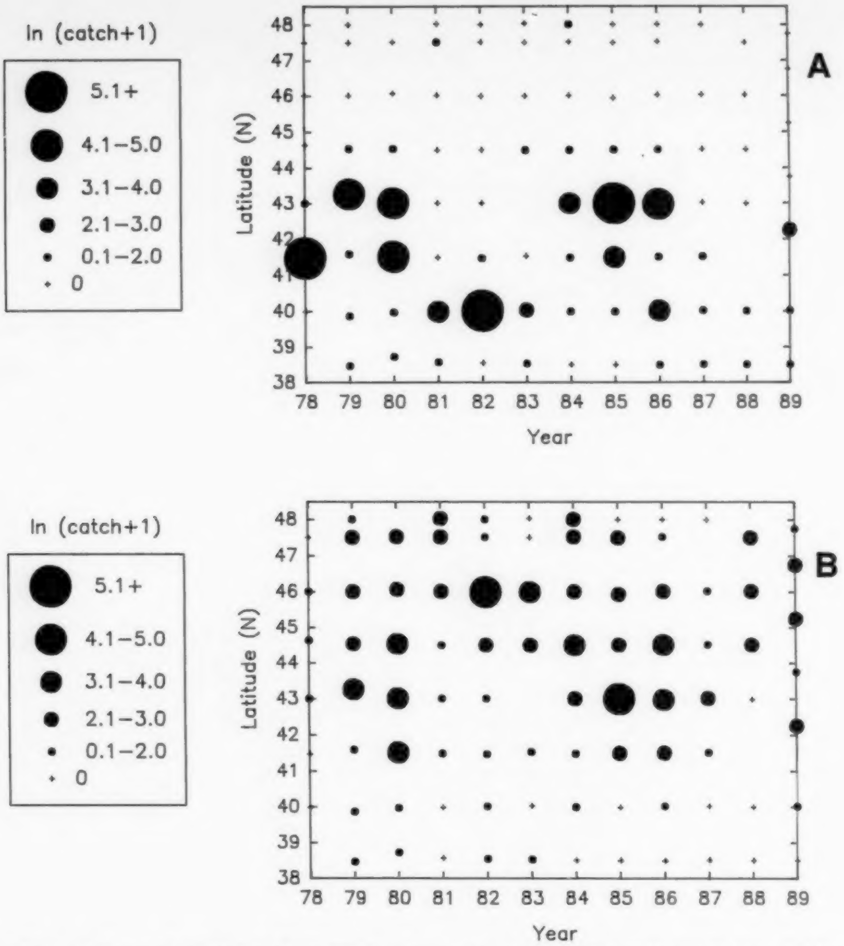

FIG. 11. Interannual variability along 175°E in the catches of (A) small (≤330 mm FL) and (B) large (>330 mm FL) pomfret, (C) sea surface temperature, (D) surface salinity, and (E) dynamic height of the sea surface relative to 700 m, the Subarctic Boundary (solid line) and the boundary between the Transitional and Central Subarctic Domains (dashed line). Stations along 175°E ± 2° of longitude are included. (*Fig. 11 concluded next page*)

with lower surface salinity, as expected in more northerly subarctic water. We found both large and small fish at higher temperatures (Fig. 10) than did Wada and Murata, probably because their collections were made in June whereas ours were made mainly in July.

Surface temperatures where pomfret were caught ranged from about 8° to 22°C, which is greater than the range reported by Hanavan and Tanonaka (1959), Neave and Hanavan (1960), and Hitz and French (1965). Machidori and Nakamura (1971) and Wada and Murata (1985) considered the 8.5–9.0°C isotherms to be close to the northern limit of distribution. We suggest that a surface temperature of 8.0°C is the thermal minimum for the occurrence of large Pacific pomfret. The southern limit of Pacific pomfret is associated with the 21°C isotherm (Mead 1972; Seki and Bigelow 1993), although we found small pomfret in water close to 22°C (Fig. 10).

Interannual trends in abundance

Interannual variations in the catches of small and large pomfret in the standard net were compared for the two most complete north-south transects, 175°E and 170°E, 1978–89.

The number of small pomfret caught at different latitudes along 175°E varied from year to year. Catches extended north of 42°N during 1978 through 1980, retreated south of 42°N in 1981 and 1982 (except one fish at 47°30′N), and then moved to the north of 44°N during 1983 through 1986 and to the south of 43°N in 1987 and 1988 (Fig. 11A). These trends generally conform to variations in sea surface temperatures. Temperatures were warm north of 42°N during northern excursions of small pomfret and cool during the intervening years when abundances were high to the south (Fig. 11C). The 13–14° isotherms generally defined the northern limits of small pomfret along this transect during July. Surface salinity (Fig. 11D) and dynamic height (Fig. 11E) did not correlate as well as temperature with catch rates. High catch rates of small pomfret were usually located north of the 34 ‰ isohaline, either at the surface or where it was vertical at 100 m (i.e., the Subarctic Boundary), and south of the northern boundary of the Transitional Domain as indicated by the salinity

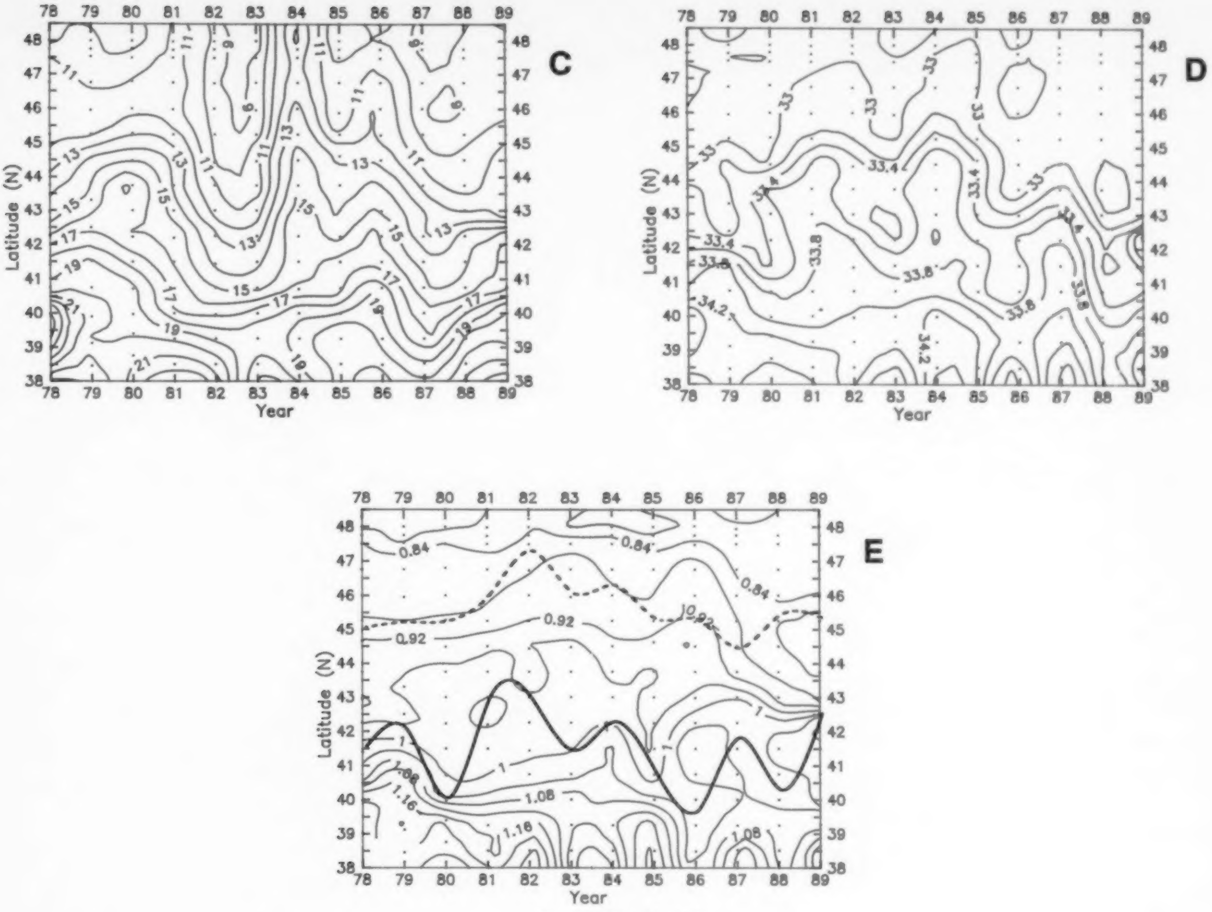

FIG. 11. (*Concluded*)

front centered between 33.2 and 33.4 ‰. During 1988 and 1989, when low surface salinities dipped to the south and strong currents were located at mid-latitudes (42–43°N) the catches of small pomfret were low at all latitudes.

Large pomfret along 175°E were distributed much farther to the north than small pomfret (Fig. 11A,B). However, both large and small pomfret were abundant at the same latitudes north of 42°N during 1980 and 1985–86. During 1982 and 1983, on the other hand, when small fish retreated to the south and sea surface temperatures declined, the highest catches of large pomfret were made at 46°N, in waters where surface temperatures were 9–10°C (Fig. 11C). This suggests that large pomfret are more eurythermal than small pomfret. Large pomfret often were common north of the front between the Transitional and Central Subarctic domains, and within the salinity front and region of accelerated currents (Fig. 11D,E).

Catch rates of small pomfret along 170°E were highest between 40° and 42°N during 1981–82 and 1984–85 (Fig. 12A), when warm surface temperatures bulged northward (Fig. 12C) and when the Subarctic Boundary extended northward (Fig. 12D). Catches were low during 1983, when low sea surface temperatures (13°C) intruded south of 41°N (Fig. 12C). Catches were generally low at all latitudes during 1986–1989, a period when unusual ocean conditions were not evident. These interannual trends have similarities to those at 175°E. Again, small pomfret were confined to waters south of the salinity front and accelerated currents associated with the northern boundary of the Transitional Domain.

High catches of large pomfret extended farthest to the north during 1984 (Fig. 12B), when warm surface waters (>10°C) occurred as far north as 47°30′N (Fig. 12C). Wada and Murata (1985) also found interannual variations in the size distribution of pomfret off northern Japan. In some years, such as 1980, the small size mode predominated in the catches. Fluctuations in the relative abundance of small pomfret suggest year-to-year variations in the year-class strength and recruitment of 0-age pomfret.

Catches in the Gulf of Alaska

Small pomfret were much less common in the eastern than the western North Pacific during July. In contrast with transects to the west, distinct modes of small pomfret were evident from size distributions of only four sets between 38°–42°30′N along 155°W (Fig. 4). Modes of small fish were not evident in the numerous sets farther to the east in the Gulf of Alaska, although a few small pomfret sometimes made up the tail end of the size distribution of the large mode.

High catches of large-sized pomfret were made during July in the Gulf of Alaska, especially from 48°–50°N along 145°W, from

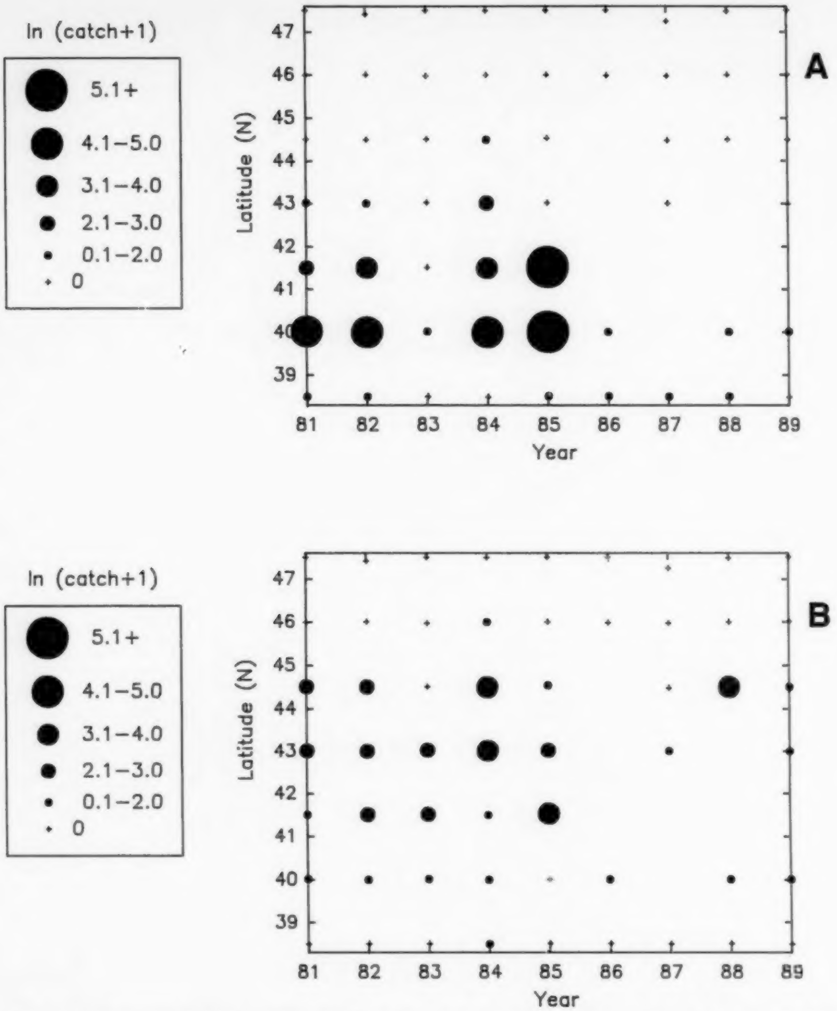

FIG. 12. Interannual variability along 170°E in the catches of (A) small (≤330 mm FL) and (B) large pomfret (>330 mm FL), (C) sea surface temperature, (D) surface salinity, and (E) dynamic height of the sea surface relative to 700 m and the Subarctic Boundary (solid line) and the boundary between the Transitional and Central Subarctic Domains (dashed line). Stations along 170°E ± 2 longitude were included. (*Fig. 12 concluded next page*)

136°–141°W along 55°N, and 52°–54°N along the coast of British Columbia (Fig. 13). Large pomfret were found in all but one set east of 141°W. The higher catches of pomfret in the eastern than the western Gulf of Alaska north of 50°N were associated with relatively high temperatures compared to waters at the same latitude to the west (11.4–12.0° vs. 8.7–10.7°C during 1982 and 13.6° vs. 10.4–11.8°C during 1983 along 55°N). These relatively warm temperatures in the eastern Gulf of Alaska may be associated with the Alaska Current.

Although sea surface temperatures in the region of low catches were lower than those farther to the east, they were always above the thermal minimum for large fish (Fig. 10). Surface temperatures at stations of zero catches were below 9°C at only 9 of 44 stations. These low or zero pomfret catches occurred in the region of the Alaska Stream at the northern stations along 155°W and where isotherms dome near the center of the Alaska Gyre (Ridge Domain) farther to the east (see Pearcy et al. 1988). Hitz and French (1965) also caught few pomfret in numerous gillnet and purse seine sets between 140°–149°W and north of 54°N in the Gulf of Alaska, but they did catch pomfret north of 55°N along 150°W. These trends suggest a possible discontinuity in the distribution of Pacific pomfret between the eastern and western Gulf of Alaska.

III. Patchiness within Gill Nets

Coefficients of dispersion of the number of pomfret caught in

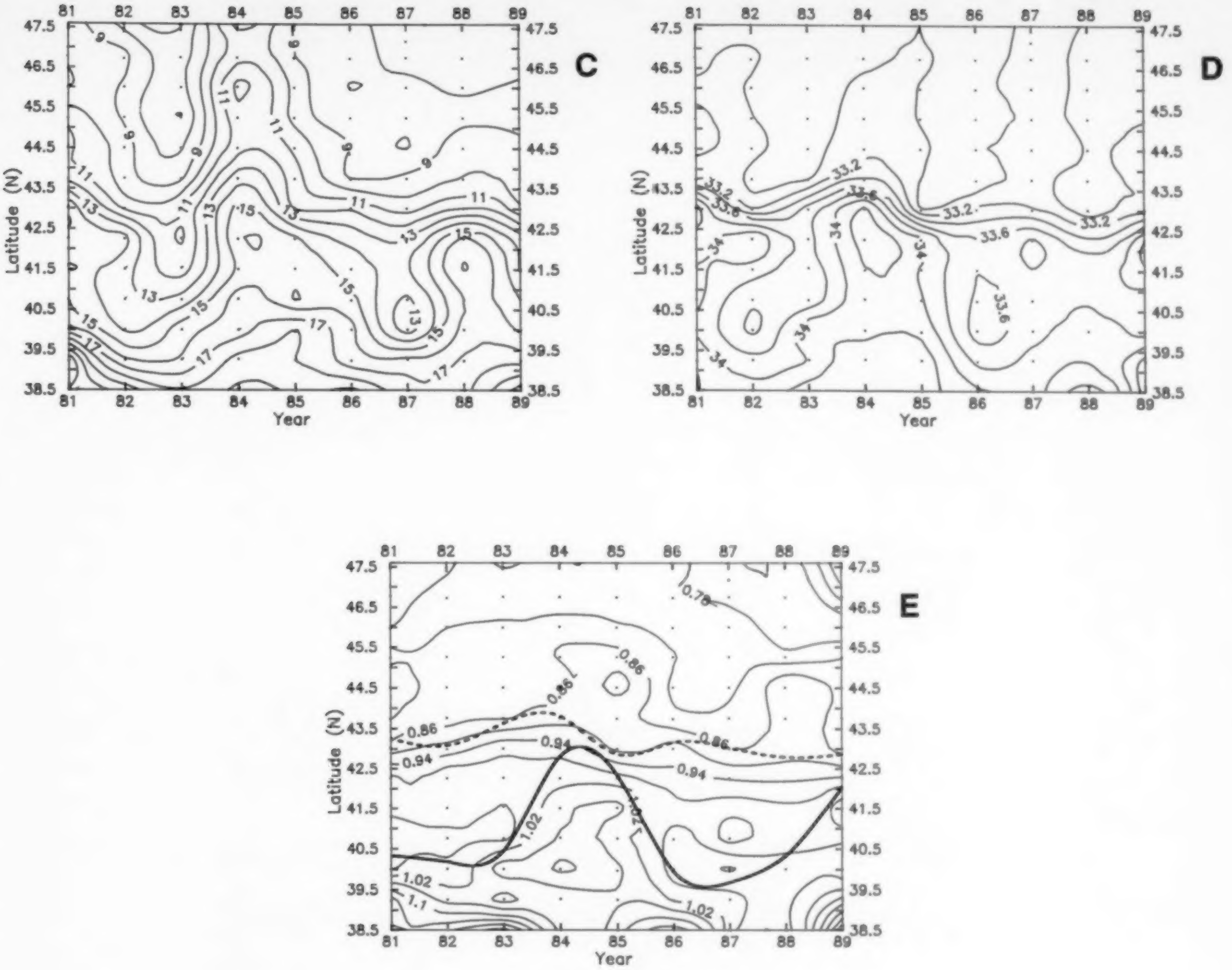

FIG. 12. (*Concluded*)

contiguous tans of the same mesh were, with one exception, close to 1.0 (0.87–3.0), for 26 comparisons. Low coefficients indicate that pomfret did not form compact aggregations or schools on a scale of a kilometer or less, but were randomly dispersed near the surface at night when they were entangled in the gill nets. Interception of many small schools at different times during the night could increase the impression of randomness. Conversely, patchiness could occur at large spatial scales that would not be detected.

IV. Age and Growth

Ages of 184 Pacific pomfret, from 211 to 500 mm FL, ranged from 0+ (young-of-the-year) to 6+ years (fish in the seventh year of life) based on otoliths. All otoliths had an opaque band of relatively rapid growth along the edge, as expected because fish were collected during summer. This was reported as "+" following the estimated age. The age of small fish was estimated from 49 whole otoliths, and confirmed by 18 sagittal or transverse sections. Ages of young-of-the-year Pacific pomfret estimated from sections and from whole otoliths did not differ. All otoliths having a surface age over 2 yr were sectioned (Fig. 14).

Pacific pomfret grow rapidly during their first 2 yr of life (Fig. 15). Growth was described with a von Bertalanffy function:

$$l_t = L_\infty (1 - e^{-k(t-t_0)}),$$

where L_∞ was estimated at 468 mm FL, k was 0.76 and t_0 was −0.52 yr. Lengths of our 6+ yr-old fish (i.e., 494 and 500 mm FL) were above the asymptote of the fitted curve, which underestimated the maximum length of pomfret. Although Pinckard (1957) suggested that Pacific pomfret reach a total length of 1220 mm, the largest previously reported sizes were 500–550 mm FL (Wada and Murata 1985). In this study, only 26 fish >500 mm FL and three fish >600 mm FL were measured. The largest was 614 mm FL, suggesting a maximum size of about 600–620 mm FL.

Our estimates of size-specific age agree with those based on annuli-like structures on scales from the caudal peduncle through age 3 (Shimazaki 1989). He reported fork lengths of 180–240 mm for age 0+, rapid growth to 360–400 mm by age 1+, and then slower growth to 380–480 mm for fish of age groups 2 and 3. However, he did not find annuli-like patterns on

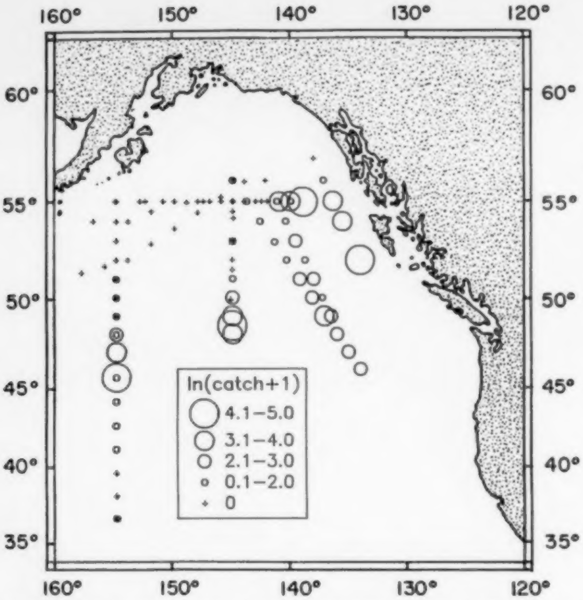

FIG. 13. Catches of large pomfret in the standard net in the Gulf of Alaska for all years.

TABLE 1. Food items identified from the stomachs of Pacific pomfret in the Gulf of Alaska and along 180°.

	Gulf of Alaska	180°
Euphausiids		
Thysanoessa spinifera	×	
Euphausia pacifica	×	
Gnathophausia	×	
Amphipods		
Cystisoma	×	×
Phronima	×	×
Themisto	×	
Shrimp		
Sergestes similis	×	
Barnacles		
Lepas		×
Pteropods		
Euclio		×
Heteropods	×	
Carinaria	×	×
Squids		
Berryteuthis anonychus	×	
Gonatus	×	×
Chiroteuthis	×	
Onychoteuthis	×	
Fishes		
Tarletonbeania	×	×
Myctophids		×
Lestidiops ringens	×	
Hemilepidotus	×	
Pleurogrammus monopterygius		×
Cololabis saira		×
Sebastes	×	

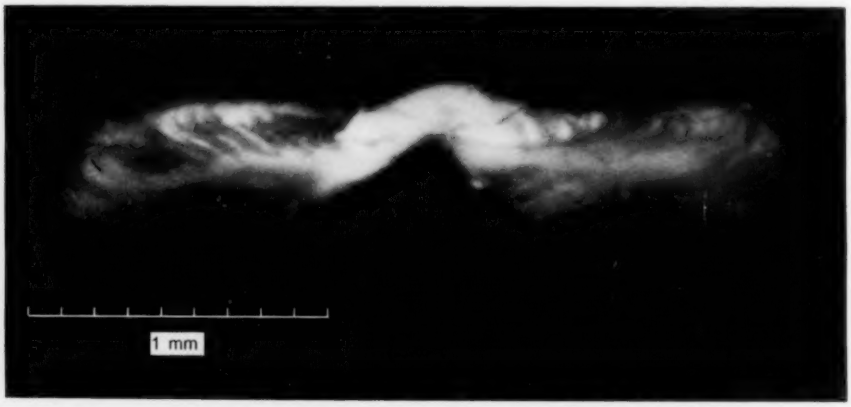

FIG. 14. Transverse section of an otolith of a Pacific pomfret, 494 mm FL. Age is estimated at 6+ yr, each yearly increment denoted by a dark line.

scales indicating age groups older than 3 yr, or minute growth increments in otolith sections indicating ages over 2 yr. Thus Shimazaki's estimates suggest that Pacific pomfret grow rapidly to 400–500 mm FL in less that four years, similar to our estimates based on otoliths.

Both our estimates and Shimazaki's differ from the much slower growth suggested by Machidori and Nakamura (1971). From otoliths and length frequencies they estimated that fish of two modal fork lengths groups, 350 and 405 mm, were age 4 and 5, respectively. Thus, we explain the absence of intermediate size modes (290–350 mm FL, Fig. 2) of Pacific pomfret during spring and summer surveys, ascribed previously to inadequate areal coverage or selectivity of gear (see Wada and Murata 1985), by the rapid growth of age 0+ fish to a large mode of slower growing fish, with intermediate sizes (300–350 mm FL) present only in the fall and winter. Based on our growth curve (Fig. 15), small fish (≤330 mm FL) in our catches were age 0+ and large fish (331–500 mm FL) were ages 1+ to 6+.

The number of fine-scale increments in sagittal sections of otoliths corroborate the rapid growth of young pomfret. Otoliths

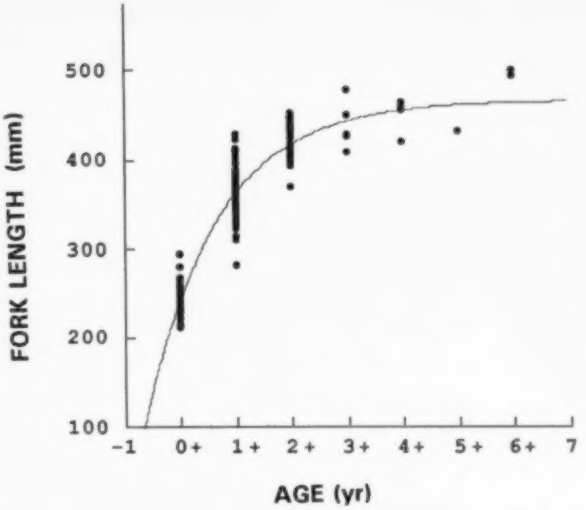

FIG. 15. Estimated age vs. fork length for Pacific pomfret. The line is a von Bertalanffy growth equation ($l_t = L_\infty (1 - e^{-k(t-t_0)})$) fitted to these estimates.

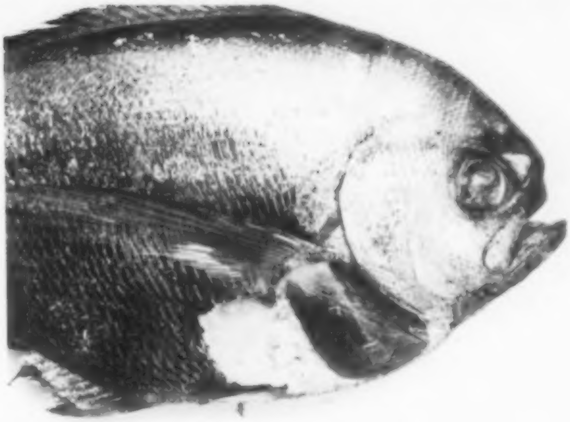

FIG. 16. Photograph of pomfret with skin removed from pectoral girdle and posterior area showing the dark coloration of the musculature of the lateral muscles associated with the pectoral fin.

from 0+ fish (200–260 mm FL) had 130–221 increments. Number of increments was positively correlated with fork length ($r = 0.63$). Assuming these are daily increments, hatch dates ranged from January to April. This supports the findings of Shimazaki (1989) that small pomfret (200–250 mm FL) caught in late July had less than 160-min growth increments, indicating than they hatched during February, again assuming that these increments are daily. Similarly, larval and juvenile pomfret caught in early March near the Hawaiian Archipelago were estimated to have hatched during January based on otolith microstructure (Seki and Bigelow 1993). It should be noted that none of the methods for ageing Pacific pomfret has been validated.

Rapid growth early in the life history of pomfret is similar to that of other epipelagic fishes, such as juvenile sablefish (*Anoplopoma fimbria*), a subarctic fish that is pelagic during its first year of life (Boehlert and Yoklavich 1985). Albacore (*Thunnus alalunga*), a highly migratory epipelagic fish of transitional waters, also has rapid growth during the first three years of life (Beamish 1981).

V. Food Habits

Pacific pomfret prey include a variety of pelagic crustaceans, mollusks, and fishes (Table 1). Gonatid squids were common in stomachs collected in the Gulf of Alaska where they dominated the stomach contents at 24 of 28 stations and usually comprised 75% or more of the prey (Table 2A,B,C,D,G). When squids were principal prey, the percentage of empty stomachs was usually low and stomach fullness was high.

Small *Gonatus* spp. squids (dorsal mantle length-DML <60 mm) were most important in the northern Gulf of Alaska where cold, saline waters protrude toward the surface (i.e., the Ridge Domain of Favorite et al. (1976)) and waters of the Alaska Current (55–53°N), whereas larger (DML >70–80 mm) *Berryteuthis anonychus* comprised most of the squid biomass consumed farther to the south in the region of the Subarctic Current (52–49°N). This species was the primary prey and contributed to the high stomach fullness of pomfret, as well as salmonids (Pearcy et al. 1988), in this region. Even farther to the south (48–44°N), in the Transitional Domain, other small squids (often gonatids) were present in pomfret stomachs but were of lesser importance. Gonatid squids were identified from pomfret caught along 180°, but no *B. anonychus* were found.

In our study, euphausiids were the major prey for large pomfret only at one station (50°N) in the Gulf of Alaska and at two stations along 180° (Table 2E,F,G). Pteropods were only found in fish from stations along 180°, but are sometimes important as food for epipelagic fishes in the subarctic eastern Pacific (Pearcy 1991). Among fishes found in pomfret stomachs, the myctophid *Tarletonbeania crenularis* occurred most frequently in the Gulf of Alaska (north of 45°N) and along 180°. The slender barracudina, *Lestidiops ringens*, occurred only in stomachs from the Gulf of Alaska, and the Pacific saury (*Cololabis saira*) was only found along 180° (Table 1).

Other investigators found that squids were important prey for large pomfret in the Gulf of Alaska, as elsewhere in the Central Subarctic Pacific (Pinckard 1957; Machidori and Nakamura 1971; Wada and Murata 1985; Shimazaki 1989). Shimazaki and Nakamura (1981) reported that squids and fishes were the most frequent food items for large pomfret during most months of the year. Kubodera and Shimazaki (1989) found that the gonatids (*Gonatus middendorffi*, *G. berryi*, and *G. pyros*) were more numerous than *B. anonychus* in stomachs of large pomfret caught west of 175°W. This is explained by the more easterly distribution of *B. anonychus* in the subarctic Pacific (Kubodera and Jefferts 1984; Kubodera and Shimazaki 1989).

Crustaceans were important prey for small pomfret off Japan during June (Wada and Murata 1985) and for large pomfret during the winter south of the Subarctic Boundary (Shimazaki and Nakamura 1981; Seki and Bigelow 1993). Euphausiids were important in the spring when other prey items were less abundant (Shimazaki 1989).

Shimazaki and Nakamura (1981) found that stomach fullness of Pacific pomfret was highest in the Subarctic Domain, where the catch per effort in gill nets was the highest, and decreased markedly to the south in the transition and subtropic regions. This high availability of pomfret prey in northern waters during the summer results in a 30% increase in body weight compared to that in the winter (Shimazaki 1989). Pomfret not only take advantage of increased secondary production of food in subarctic waters during the summer, but also follow small migratory fishes such as Pacific saury, sardine, and Pacific mackerel northward during the summer (Taniguchi 1981; Shimazaki 1986).

In the western Pacific, fishes rather than squids are often reported as the most important prey taxon for pomfret. Here the Japanese sardine (*Sardinops melanostictus*) was often the principal prey of large pomfret (Kohno 1983; Wada and Murata 1985), but in 1991, following a decline in Japanese sardine, pomfret fed on Japanese anchovy (*Engraulis japonicus*) and squids (Wada and Honda 1992), demonstrating their opportunistic feeding behavior.

VI. Parasites, Predators, and Populations

The only large parasite found in the flesh of pomfret was the larval stage of the trypanorynch tapeworm *Molicula* sp., that burrows into the muscle of the fish and eventually reproduces in the digestive tract of its final host. Two and one-half times more larval *Molicula* were found in pomfret caught in the western than the eastern Pacific (mean (std. dev.) 4.3 (2.53)) tapeworms per fish vs. 1.7 (1.4); $P < 0.005$).

These observations on parasites are important for two reasons. First, because all adult trypanorynchs occur only in elasmobranchs (Rohde 1984), sharks may be the major predator of Pacific pomfret. The blue shark and the Pacific pomfret have broadly overlapping distributions in the Transitional Domain of the subarctic Pacific during the summer (Mishima 1981; Pearcy 1991). Pomfret remains have been reported in the stomachs of the blue shark (*Prionace glauca*) in the Gulf of Alaska (LeBrasseur 1964), and in the Subarctic Frontal Zone north of the Hawaiian Islands (Seki 1993).

Second, this difference in parasite loads, as well as postulated differences in the spawning season of Pacific pomfret in the eastern and western Pacific, the absence of distinct modes of small pomfret in the eastern Gulf of Alaska, and the hiatus in distribution of large pomfret in the central Gulf of Alaska, all suggest geographic differences in stock structure.

Yoon and Shimazaki (1981) reported seasonal peaks in the gonosomatic indices that suggested the main spawning season of Pacific pomfret was early June and July between 140–177°W and during the winter and early spring (December–April) farther to the east (160–170°E) (see also Shimazaki 1989; Seki and Mundy 1991). The few data on the occurrence of larval and juvenile pomfret, however, indicate that the subtropic region in the mid-Pacific (29°47′N, 179°E) is a winter spawning area (Seki and Mundy 1991). At 28°N, 155°W, Loeb (1979) collected 49 larval pomfret during January–February and three during September, again suggesting primarily winter spawning in this region. These studies suggest that pomfret spawn during the winter in the central and eastern Pacific, not during June and July as postulated by Yoon and Shimazaki (1981).

Farther to the east in the southern region of the California Current (ca. 27–35°N and east of 125°W), *Brama* spp. larvae are rare (Stevens et al. 1990), but *B. japonica* larvae were collected during most months of the year, indicating year-around spawning, with the highest average abundance during October–December (G. Moser personal communication). Four of the five Pacific pomfret caught by Berry and Perkins (1966) off California (30°07′–34°47′N, 124°31′–129°16′W) appeared to be from spawning in the winter (15.5 mm standard length (SL) in March and 215–220 mm SL (similar to our age 0+) during August). However another specimen of 52.5 mm SL was collected during August, suggesting spring spawning. Thus it seems premature to conclude that Pacific pomfret has different spawning seasons in the eastern and western Pacific. We agree with Shimazaki (1989) that more research is needed on the seasonality of spawning and occurrence of eggs and larvae of Pacific pomfret to determine the existence of separate populations in the North Pacific.

VII. Swimming Behavior

During several cruises of the Oshoro Maru, it was noted that Pacific pomfret caught in gill nets set overnight and retrieved during the early morning were always dead, whereas other fishes (e.g., salmonids), were sometimes alive. This observation led to the hypothesis that Pacific pomfret swim continuously to ventilate their gills, and that entanglement in gill nets interferes with respiration and causes death. To test this idea, live pomfret were observed in tanks with running sea water aboard the ship.

Pacific pomfret (35–40 cm FL) swam continuously in tanks at speeds of about one body length per second. Propulsion was exclusively by their long, falcate pectoral fins (34–38% of standard length; Mead 1972) that flapped in unison about once per second. The forces of buoyancy and lift were about equal because the axis of body motion was nearly horizontal during pectoral swimming. The pectorals were also used to dive rapidly and to change vertical position in the tank. The caudal fin was used to accelerate and to turn at the ends of the tank. Bending of the relatively rigid dorsal and anal fins was also observed during turns, but these fins did not flap. Pomfret were very maneuverable and seldom collided with the walls of the tank. Their maneuverability in nature may assist in capturing squids which accelerate rapidly.

At swimming speeds of about 30–40 cm/s, the mouth was open and the opercle was slightly open. Opercular pumping was not seen. These observations indicate ramjet ventilation of the gills. Gape distance appeared to vary inversely with swimming speed. However, two fish that were on their sides on the bottom of the tank remained alive after 24 h and used opercular pumping to ventilate their gills. This suggests that ramjet ventilation is not obligatory, and that the death of Pacific pomfret in gill nets was due to interference with opercular movements, as well as prevention of ramjet ventilation, their normal means of gill ventilation.

Dissection of several fish at sea revealed that both the abductor and adductor muscles of the pectoral fin consisted of dark red muscle fibers, presumably enriched in capillaries and myoglobin. Red muscle is used for continuous propulsion in other fishes (Webb 1975). These dark red muscles contrasted with the white muscle fibers seen throughout the rest of the body (Fig. 16). No red muscle was associated with the lateral muscle mass of the body as in tunas and other fast swimming pelagic fishes.

Pectoral fin muscles of one specimen (355 mm FL, 759 g total weight) were dissected and weighed. The paired abductors (superficialis and profundus) located on the lateral face of the pectoral girdle (see Winterbottom 1974 for muscle morphology)

TABLE 2. Food habits of pomfret collected in the Northeast Pacific from the T/V *Oshoro Maru*, 1980–87. (A) percent of wet-preserved weights of taxa, (B) percent frequency of occurrence, and (C–G) percent by volume.

A. 1980, along 145°W

	Latitude north	
	50°	48°30′
Euphausiids	0	2
Amphipods	0	T
Squids	86	91
Fishes	4	3
Unidentified	10	5
No. examined	10	50
% empty	10	20

D. 1985, along 155°W

	Latitude north								
	48°	47°	45°30′	44°	42°30′	41°	39°30′	38°	36°30′
Squids	98	100	95	100	100	5			58
Fishes	2	0	5	0	0	21			38
Other	0	0	0	0	0	74			5
No. examined	38	20	30	6	1	25	0	0	6
% empty	0	5	10	17	0	40			33
Fullness	2.9	3.0	2.2	2.2	1.0	1.3			2.0

B. 1981, along 145°W

	Latitude north		
	51°	50°	49°
Amphipods	0	5	8
Squids	88	69	90
Fishes	38	0	6
No. examined	8	42	50
% empty	0	12	2
Fullness	1	2	2

E. 1986, along 180°

	Latitude north					
	37°	38°	39°	40°	41°	42°
Euphausiids	11	24	26	59	34	4
Amphipods	0	4	2	<1	16	8
Squids	11	15	1	7	17	35
Fishes	78	38	36	23	16	46
Other	0	18	35	11	18	6
No. examined	26	23	31	27	28	27
% empty	34	4	22	4	11	7
Fullness	2.4	1.9	2.1	1.9	1.3	1.8

C. 1984, along 155°W

	Latitude north				
	49°	48°	47°	45°	44°
Euphausiids	0	0	<1	0	0
Amphipods	0	0	7	15	2
Squids	92	64	80	73	23
Fishes	8	36	5	7	50
Other	0	0	7	5	25
No. examined	14	25	30	30	33
% empty	14	60	13	33	36
Fullness	3.43	0.8	2.53	0.5	1.5

F. 1987, along 180°

	Latitude north			
	39°	40°	41°	42°
Euphausiids	<1	53	12	11
Amphipods	0	9	3	0
Squids	12	<1	25	74
Fishes	72	37	36	10
Other	5	9	25	5
No. examined	20	58	39	10
% empty	60	16	48	0

G. 1987, on a diagonal from 56°N to 45°59′N

	Latitude north, longitude west										
	56°00′ 145°00′	55°00′ 143°48′	54°00′ 142°38′	52°27′ 141°23′	52°60′ 140°20′	51°00′ 139°12′	50°00′ 138°08′	49°00′ 137°05′	48°00′ 136°00′	47°00′ 135°00′	45°59′ 133°58′
Euphausiids	0	27	0	<1	0	0	60	0	0	<1	0
Squids	47	68	95	66	100	75	32	62	74	97	91
Fishes	53	5	2	5	0	25	8	16	12	2	9
Other	0	0	3	29	0	0	0	22	14	<1	0
No. examined	3	23	28	30	11	29	30	35	31	31	32
% empty	0	17	4	30	18	58	60	17	16	26	66
Fullness	1.0	1.7	1.8	1.3	2.9	1.6	1.5	2.3	1.6	1.2	1.4

had about the same mass as the adductor muscles on the medial face of the pectoral girdle. The combined weight of these muscles (9.8 g) from one pectoral girdle was 4.5% of the combined white (210 g) and red muscles mass from the same side of the body. This is about the same ratio of red muscle to total musculature reported for the labrid *Oxyjulis californica*, that relies on pectoral fins for normal swimming (Rosenblatt and Johnson 1976), but less than the ratio of red to white muscles found in myotomal musculature of rainbow trout (*Oncorhynchus mykiss*) (Webb 1971) and of red muscle to body mass in most of the scombrids studied by Magnuson (1973). It is far less than the ratio reported for the ocean opah (*Lampris guttatus*), that swims with its comparatively short pectoral fins and has large red adductor and abductor muscles comprising 37% of the total propulsive musculature (Rosenblatt and Johnson 1976).

Conclusions

Pacific pomfret has attracted attention as an underutilized resource because of its high abundance in fisheries for other species. Often it is the dominant nonsalmonid fish in gill net and purse seine catches in the subarctic region during the summer (Neave and Hanavan 1960; Hitz and French 1965; Machidori and Nakamura 1971; Yatsu et al. 1993). High abundance throughout much of the epipelagic waters of the North Pacific, rapid growth rate, and segregation of large and small individuals during northward migrations are favorable characteristics of a potentially productive fishery. In addition, we found no decrease in either catch per standard set of large pomfret or mean size of pomfret during our study that was coincident with the large-scale drift net fishery for flying squid. This indicates that the population of Pacific pomfret had not been adversely affected by its incidental catch in the flying squid fishery.

Although much is known about the biology of pomfret (see Shimazaki 1989), important features of their biology and ecology need to be evaluated. Differences in parasite loads and age structure suggest different stocks in the eastern and western North Pacific. The discreteness of stocks is an important management consideration and should be resolved, possibly by biochemical or genetic studies or larval surveys, before intensive exploitation.

Reliable methods of ageing need to be further developed, including an age-specific study to validate growth increment periodicity. Age determination is vital to estimates of growth, mortality, age at first spawning, and ages of large and small fish during the summer season. Information on reproduction in the Northeast Pacific is also needed.

Little is known about the early life history of Pacific pomfret, and nothing about factors affecting survival and year-class success. Better understanding also is needed on the role of Pacific pomfret in the North Pacific pelagic ecosystem and how a fishery would affect other species, including salmonids.

Finally, fishing techniques to catch pomfret may be problematical. Drift gill nets are not an acceptable option unless bycatch of other species is minimal. To target pomfret with purse seines or trawls, more information is needed on gear selection, schooling behavior, and oceanographic and geographic factors that may result in dense, available concentrations.

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Spatial Variation of Crustacean Plankton in Lakes of Different Size[1]

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Patalas, K., and A. Salki. 1993. Spatial variation of crustacean plankton in lakes of different size. Can. J. Fish. Aquat. Sci. 50: 2626–2640.

The patterns of spatial distribution of planktonic crustaceans changed with increasing lake size. Greatest abundances were often found offshore in smaller lakes but nearshore in larger lakes. Interstation similarity of plankton, measured with Jaccard's and Renkonen's indices, was higher in small- to middle-sized lakes and lower in very small and very large lakes, indicating that mechanisms existed at both ends of the size spectrum which prevented plankton from mixing horizontally. The information content of a single central lake sample was evaluated against a lake average from 9–50 stations. To capture at least 80% of the species present, one station was sufficient only in smaller lakes, three to six stations were needed in the smallest and medium-sized lakes, and more than nine stations were needed in the largest lake. The single central station in small- and medium-sized lakes represented average total plankton abundance and dominant species relatively well but underestimated rare species. In larger lakes, lake average plankton was not well characterized by a single station. In Lake Superior, the central station reflected the offshore but not the nearshore community. Neither plankton abundance nor the number of species appeared related to lake size in the series of lakes investigated.

La forme de la répartition spatiale des crustacés planctoniques a changé avec la superficie des lacs. Dans les petits lacs, on a souvent trouvé les crustacés en plus grande abondance au large, mais près du rivage dans les lacs plus grands. La similitude du plancton entre stations, mesurée selon les indices de Jaccard et de Renkonen, est supérieure dans les lacs de taille petite à moyenne, et inférieure dans les très petits et les très grands lacs; cela indique qu'il existe des mécanismes aux deux extrêmes de taille des lacs qui agissent de manière à ce que le plancton ne se mélange pas sur le plan horizontal. On a comparé les renseignements livrés par des échantillons centraux uniques aux renseignements livrés par des ensembles d'échantillons provenant de 9 à 50 stations. Si l'on prend comme critère qu'au moins 80 % des espèces présentes doivent se trouver dans l'échantillon, une seule station ne suffit que dans le cas des petits lacs; il faut des échantillons de trois à six stations pour le plus petit lac et les lacs de taille moyenne, et il faut plus de neuf stations dans le cas du plus grand lac. La station centrale unique dans les lacs de petite et moyenne tailles donne une assez bonne idée de l'abondance moyenne du plancton total ainsi que de l'espèce dominante, mais sous-estime les espèces rares. Dans les grands lacs, la composition planctonique moyenne dans le lac n'est pas bien caractérisée par une station unique. Dans le lac Supérieur, la station centrale est représentative des conditions du large, mais pas de la communauté qui vit le long du rivage. Ni l'abondance plancton ni le nombre d'espèces ne semblent être en fonction de la grandeur du lac dans le cas des lacs étudiés.

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Researchers studying lake plankton often make intuitive, tacit assumptions. One common assumption, based on Hensen's (1887) original definition, is that plankton is passively drifting in water, and hence is either homogenously or randomly distributed in lakes. Naber (1933) found a random distribution in Grossen Ploener See in Germany. Ricker (1937) compared a series of replicate net hauls at a single station in Cultus Lake, B.C., with single hauls at random sites. He concluded that although patchiness was evident for most groups of plankton, there was little difference between population estimates from either one station or several stations. Langford (1938) detected a rather patchy horizontal distribution in Lake Nipissing. Berzins (1958) found that, even in a small pond, the distribution of rotifers and cladocerans was patchy and that different patterns of distribution existed for different species. A comprehensive review of literature on this topic was given by Malone and McQueen (1983). They classified patches in four categories: (I) large scale (>1.0 km in diameter), (II) small scale, caused by wind-induced water movement, (III) Langmuir circulation aggregations, and (IV) swarms, potentially caused by biotic factors. They also found distinct patches in the horizontal and vertical distribution of most cladoceran and rotifer species in two small (1 and 10 ha) Ontario kettle lakes. Pinel-Alloul and Pont (1991) studied spatial patterns of several species of copepods and cladocerans in a small Canadian Shield lake (9.2 ha). They identified fine patches (2–40 m in diameter) and larger, lake-size patches (10–380 m in diameter). Spatial heterogeneity occurred on both the fine and coarse scales, but maximal heterogeneity was observed in the vertical rather than in the horizontal axis. Neither of the spatial scales was necessarily associated with a given species group because a species group could exhibit different spatial patterns in different water bodies or even within the same lake. Environmental factors

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[1] This is one in a series of papers describing work of the Freshwater Institute Science Laboratory on a lake size series in Northwest Ontario.

TABLE 1. Lakes size, fetch, maximum depth (Fee and Hecky 1992), and number of stations.

Lake	Area (km^2)	Fetch (km)	Max. depth (m)	No. of stations	Distance between stations (km)
Lake 373	0.27	0.6	21	9	0.16
Green	0.89	1.1	18	9	0.25
Orange	1.67	1.8	28	9	0.4
Linge	7.06	3.4	22	9	0.8
Musclow	22.19	5.5	43	11	1.5
Sydney	57.48	8.5	71	10	2.5
Trout	346.9	17.0	47	20	4.5
Nipigon	4848.0	70.0	143	20	15.0
Superior	82367.0	400	403	50	50.0

such as temperature, oxygen, chlorophyll level, and wind action appeared to explain a substantial proportion of both the vertical and horizontal heterogeneity. The authors concluded, among other things, that empirical descriptions of spatial structure were of critical importance in defining scales of patterns. Patches of much larger scales, tens of kilometres in size, were observed in the North American Great lakes (Patalas 1969, 1981, 1990, 1992). de Nie et al. (1980) reported the existence of patchiness of different scales in Lake Tjeukemeer (The Netherlands) with wind considered as an important factor in reducing the patchiness.

In designing experimental strategies, plankton researchers are continually faced with the following problem: how to resolve the conflict between sampling on adequate number of stations, on the one hand, and apportioning (usually scant) resources, on the other. A useful study on this subject was published by Downing et al. (1987). They found that plankton sampling variance (S^2), based on the analysis of numerous data from the literature, increased with population density m (individuals per litre) and decreased with increased sampler volume V (litres) according to the function $S^2 = 0.745m^{1.622}V^{-0.267}$. The validity of this regression was later confirmed by Pace et al. (1991). In situations where information on distributional patterns is not available, the number of samples (n) necessary to achieve a required level of precision (p) (where $p = \mathrm{SE}/m$ and $\mathrm{SE} = s/n^{0.5}$) can be calculated as $n = 0.745\, m^{-0.378}V^{-0.267}\, p^{-2}$ (Downing et al. 1987).

Many of the systematic studies on the horizontal distribution of macroplankton were conducted on relatively small lakes and ponds (e.g., Malone and McQueen 1983; Pinel-Alloul and Pont 1991). The distinct patchiness of plankton found in such small water bodies indicates that the convenient assumption that smaller lakes tend to be more homogeneous than larger lakes is not necessarily true. Several researchers concluded that wind was one of the most important factors controlling the horizontal distribution of plankton (de Nie et al. 1980; Malone and McQueen 1983; Patalas 1990; Pinel-Alloul and Pont 1991). Because vertical and horizontal transport and mixing of water by wind are a function of lake size (Patalas 1981), one would expect that the horizontal distribution of plankton would also vary with this parameter. Accordingly, the main purpose of this investigation was to (a) study the effect of lake size on spatial patterns and composition of plankton and (b) test empirically the effect of limited numbers of stations on the lake average estimate.

This paper is part of a concerted effort known as the Northwest Ontario Lake Size Series (NOLSS) to discover how lake size per se influences limnological phenomena. The study rationale of NOLSS as well as the limnological characteristics of the study lakes were described in detail by Fee and Hecky (1992). The nine lakes were chosen for their geological, hydrological, and morphological similarity (Canadian Shield geology; water renewal time >5 yr; fully stratified in summer) and also for the wide range in surface area (0.27–82 367 km^2).

Methods

Zooplankton samples were collected from Lake Superior during a single sampling cruise during August 18–28, 1968, and from the remaining lakes from July 21 to August 28, 1987 and 1988. Sampling duration ranged from a few hours on the smaller lakes to 10 d on Lake Superior. The sampling was carried out during the period of time when the maximum number of planktonic species as well as the maximum horizontal heterogeneity could be expected prior to autumn cooling and increased wind action (Patalas and Salki 1992). Vertical tows were taken with Wisconsin nets (mouth opening 25 cm^2, mesh size 73 μm) from near the bottom to the surface in the smaller lakes and from a depth of 50 m to the surface in Lake Superior. From 500 to 2500 L of lake water was filtered in a single haul assuming a filtration efficiency of 100%. In fact the efficiency was between 45 and 85%, averaging 63%, as indicated by later experiments, so that data should be interpreted as relative densities. Samples were collected from 9–50 stations in each lake (Table 1). Stations were uniformly distributed over the entire area of each lake and the distances among them were approximately similar. Although the number of stations increased with lake size, the average distance among stations also increased, from 0.16 km in Lake 373 (the smallest) to more than 50 km in Lake Superior (the largest). Consequently, the density of stations declined dramatically from 33 to 0.0006 · km^{-2}. As most lakes were of relatively regular shape, fetch correlated well with area (Fig. 1) and was defined as the average of maximum effective length and maximum effective width (Welch 1948).

Samples were preserved in 4% formalin. Animals were identified and counted from subsamples representing between 1.5 and 5% of total sample volume using a 1-mL Sedwick-Rafter counting chamber and a 63× compound microscope. Usually from 400 to 600 specimens (range 200–1000) were counted and identified per sample. Larger and less abundant animals such as *Leptodora* and *Senecella* were enumerated in the whole or a large fraction (one quarter to one half) of the sample under a 12× dissecting microscope.

As the frequency distributions of plankton concentrations were not normal, they did not lend themselves to parametric tests. Some distributions were bi- or trimodal. Therefore, more emphasis was placed on empirical descriptions of the spatial structure and on multivariate analyses to measure the

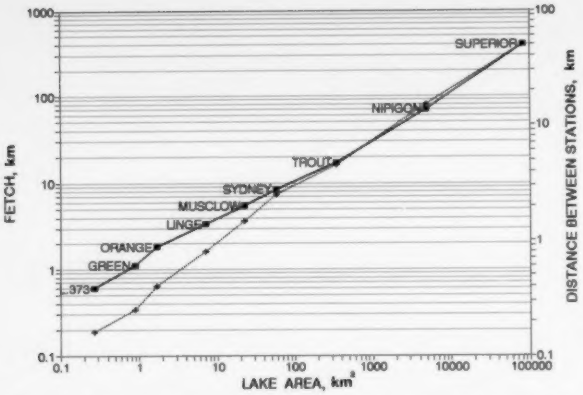

FIG. 1. Relationship between lake area, fetch (solid squares), and average distance among stations (crosses).

resemblance of species among samples taken from different stations within a lake. Similarity coefficients presented in the literature have been critically reviewed by Legendre and Legendre (1983). Two of the simplest and most informative coefficients are used in this paper: Jaccard's (1912) coefficient of community and Renkonen's (1938) percentage similarity index. Jaccard's community coefficient,

$$\frac{C}{C + A + B}$$

where A = number of species found only at station A, B = number of species found only at station B, and C = number of species common to both stations, is based on the presence or absence of species; it does not reflect the abundance of the species in two compared samples. On the other hand, Renkonen's percentage similarity index (P),

$$P_{ij} = \Sigma k \mathrm{Min}\,(Xk_i, Xk_j),$$

places emphasis on dominant species and is calculated as the sum of minimum frequencies (X) of species (k) within a sample pair of i and j. Thus, in Jaccard's coefficient, every species has the same "weight" whereas in Renkonen's index, the weight of species is proportional to its relative abundance.

Both indices range from 0, in two samples without a single common species, to 1 where two samples have all the same species and additionally in Renkonen's percentage similarity where those species occur in the same proportions. Within a well-mixed water mass, the distribution of planktonic animals would be expected to be relatively uniform and both similarity indices would tend to reach a value of 1.0 for station-to-station comparisons.

The results of similarity computations are usually presented in the form of dendrograms. A complete linkage agglomerative clustering analysis (Sørenson 1948) was used in the present study to compare lakes among themselves. In this method, two clusters can fuse only when all objects in the first cluster are linked to all objects in the second cluster (Legendre and Legendre 1983). Additionally, a constrained clustering method was applied following the procedure described by Legendre and Legendre (1984) whereby only neighbouring stations were allowed to cluster. This method was used for interpretation of the homogeneity of plankton within a lake.

Results

Spatial Distribution of Crustacean Species in Lakes

The patterns of distribution described in Fig. 2–5, based on a single sampling, can be envisioned as still photographs of a dynamic process influenced by water mass movements as well as planktonic growth and mortality. Comparison of such momentary photographs taken in a series of lakes varying in size can contribute to a better understanding of the spatial structure of lake biota. A few examples of the distribution of selected species are presented below. Of the nine lakes studied (Salki 1992), four were selected for presentation in this paper: the smallest Lake 373, the midsized Musclow Lake, the large-sized Lake Nipigon (characterized by its high degree of insularity), and at the extreme end of the size spectrum the largest Lake Superior.

Lake 373 (Fig. 2e)

Lake 373 is the smallest lake in the size series. Total numbers of crustaceans were distributed relatively uniformly throughout the lake, ranging from 15 to 35 ind. · L^{-1}, with maximum numbers found near the centre of the lake and the lowest values in the eastern bay (Fig. 2a). *Daphnia galeata mendotae* (Fig. 2b) had a tendency to occur in greater numbers (1–2.7 ind. · L^{-1}) in the main body of water, with fewer specimens found in two nearshore areas. *Diacyclops bicuspidatus thomasi* (Fig. 2c) and *Diaptomus sicilis* (Fig. 2d) constituted the most abundant component of the plankton; hence, their distributional patterns had the greatest effect on the total number of crustaceans, with the highest values in the central part of the lake.

Musclow Lake (Fig. 3e)

Total numbers of crustaceans (Fig. 3a) (9–19 ind. · L^{-1}) were relatively uniformly distributed in Musclow Lake, although the patterns of numbers of individual species were quite heterogeneous. *Acanthocyclops vernalis* (Fig. 3b) decreased gradually from 3.1 ind. · L^{-1} at the eastern shore to 0.5 ind. · L^{-1} in the western, deeper part of the lake. *Limnocalanus macrurus* (Fig. 3c) and *Diaptomus sicilis* (Fig. 3d) tended to occur in greater densities in the deepest, west-central part of the lake. Very few specimens of these two species were found at the shallower inshore stations.

Lake Nipigon (Fig. 4f)

Three distinct zones of crustacean abundance were apparent: (1) a relatively homogeneous but plankton-poor main water body (1–9 ind. · L^{-1}), (2) a plankton-rich zone along the northern shore, with densities ranging from 20 to 46 ind. · L^{-1}, and (3) an intermediate zone with 10–20 ind. · L^{-1} (Fig. 4a). Similar patterns were observed in the distribution of *Diacyclops bicuspidatus thomasi* (Fig. 4b), *Daphnia galeata mendotae* (Fig. 4c), and to a lesser extent of *Diaptomus sicilis* (Fig. 4d). These patterns closely resembled the horizontal gradient of temperature whereby warmer waters accumulated along the northwestern shore, with a gradual cooling toward the southeastern end of the lake (Fig. 4e). A wide range of temperatures (from 15 to 23.6°C) was observed in the lake during a semisynoptic survey taken during July 25–28, 1990.

Lake Superior (Fig. 5e)

In Lake Superior, the highest abundances of total crustacean plankton (20–35 ind. · L^{-1}) were found in inshore areas, particularly along the southern shore (Fig. 5a). Lowest densities, from 5 to 10 ind. · L^{-1}, were characteristic of the open-water zone. An intermediate zone with densities between 10 and 20 ind. · L^{-1}

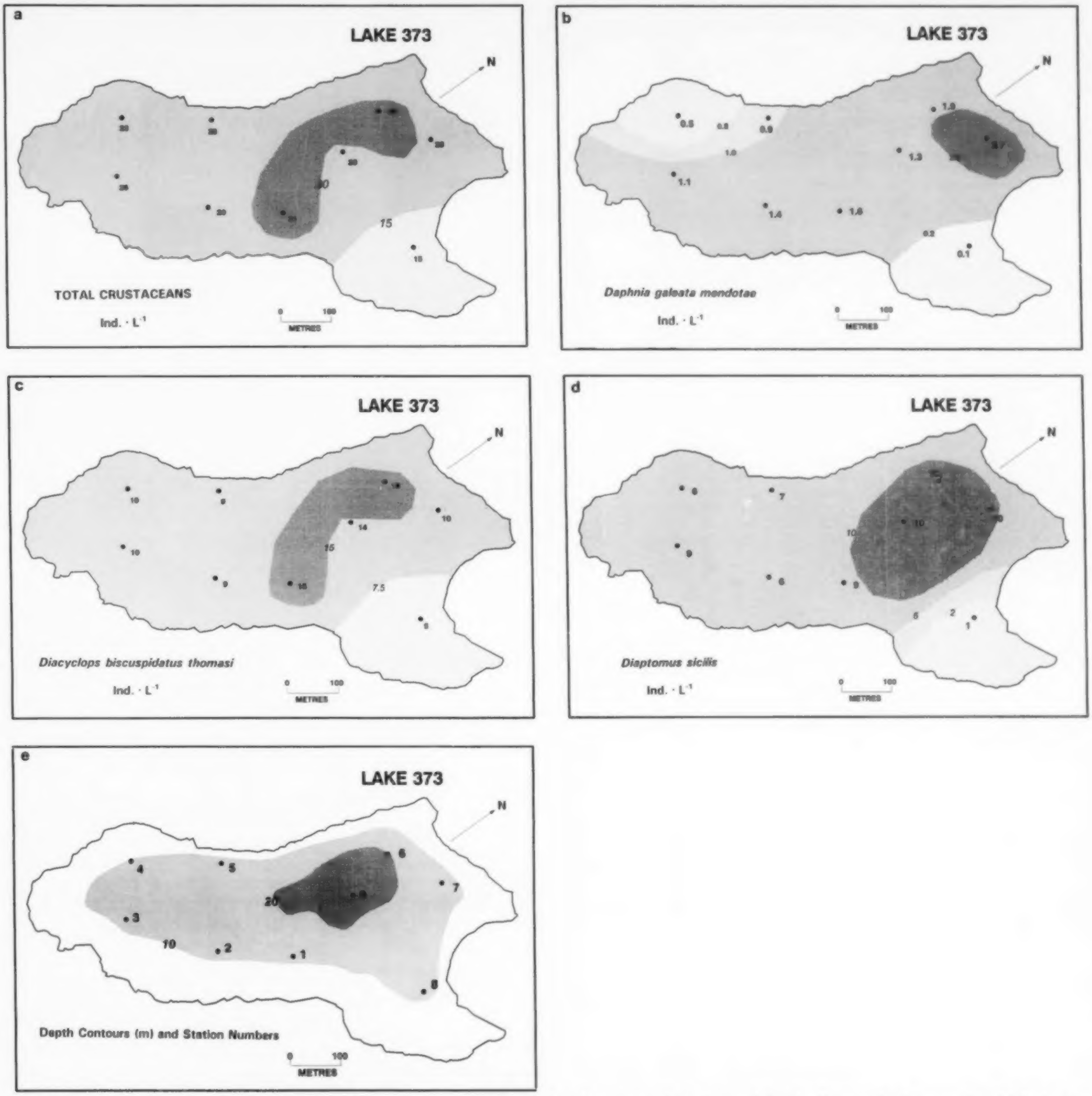
a
LAKE 373
N
TOTAL CRUSTACEANS
Ind. · L⁻¹
0 100
METRES
b
LAKE 373
N
Daphnia galeata mendotae
Ind. · L⁻¹
0 100
METRES
c
LAKE 373
N
Diacyclops biscuspidatus thomasi
Ind. · L⁻¹
0 100
METRES
d
LAKE 373
N
Diaptomus sicilis
Ind. · L⁻¹
0 100
METRES
e
LAKE 373
N
Depth Contours (m) and Station Numbers
0 100
METRES

FIG. 2. Spatial distributions in Lake 373. Numbers on the maps indicate abundance at each station and the shading marks the areas in which similar plankton densities were found. Each consecutively darker shading represents at least a doubling of plankton density (a) Total crustaceans; (b) *Daphnia galeata mendotae*; (c) *Diacyclops bicuspidatus thomasi*; (d) *Diaptomus sicilis*; (e) depth contours and sampling stations.

was observed along the western, northern, and eastern shores. These patterns reflected the thermal structure of the lake (Fig. 5b) with its prominent midlake upwelling area that coincides with the area of greatest depth (Anonymous 1970). In this area in midsummer, surface temperatures were less than 6–7°C, while, at the same time, water masses closer to shore reached 16–17°C. *Limnocalanus macrurus* (Fig. 5c) was the most abundant species in the midlake upwelling area, with only a few specimens found near shore. In contrast, *Diacyclops bicuspidatus thomasi* (Fig. 5d) was not found in the upwelling area but increased in numbers towards shore. Certain cladocerans, such as *Leptodora kindtii*, were confined to the warmest nearshore areas. Two different communities inhabited Lake Superior: calanoids (mainly *Limnocalanus macrurus* and *Diaptomus sicilis*) which dominate offshore and cyclopoids (particularly *Diacyclops bicuspidatus thomasi*) which dominate near shore. The cladocerans *Daphnia retrocurva* and *Bosmina longirostris* were also found only near shore (Patalas 1990). This dual character of the planktonic communities of Lake Superior confirms the observations of Watson and Wilson (1978) who,

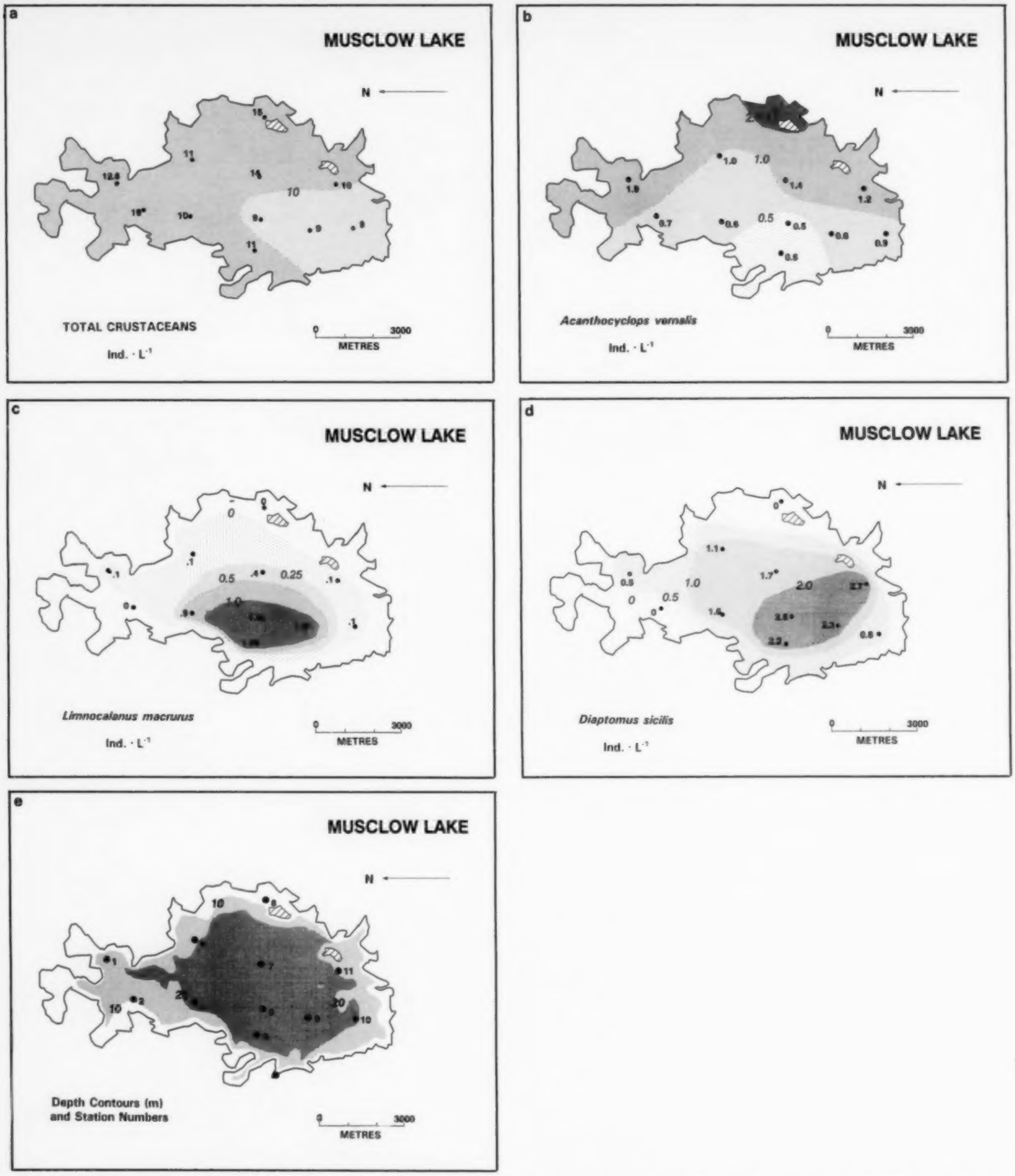
a
MUSCLOW LAKE
N
10
TOTAL CRUSTACEANS
Ind. · L⁻¹
0
3000
METRES
b
MUSCLOW LAKE
N
1.0
0.5
Acanthocyclops vernalis
Ind. · L⁻¹
0
3000
METRES
c
MUSCLOW LAKE
N
0
0.5
0.25
1.0
Limnocalanus macrurus
Ind. · L⁻¹
0
3000
METRES
d
MUSCLOW LAKE
N
1.0
0.5
0
2.0
Diaptomus sicilis
Ind. · L⁻¹
0
3000
METRES
e
MUSCLOW LAKE
N
10
20
10
20
10
Depth Contours (m)
and Station Numbers
0
3000
METRES

FIG. 3. Spatial distributions in Musclow Lake. (a) Total crustaceans; (b) *Acanthocyclops vernalis*; (c) *Limnocalanus macrurus*; (d) *Diaptomus sicilis*; (e) depth contours and sampling stations.

based on a dense network of 80 samples collected in 1973, found that the larger calanoids (*Diaptomus sicilis*, *Limnocalanus macrurus*, and *Senecella calanoides*) increased in relative abundance with distance from the shore, while cladocerans (*Bosmina longirostris*, *Daphnia galeata mendotae*) and cyclopoids (*Diacyclops bicuspidatus thomasi*) decreased.

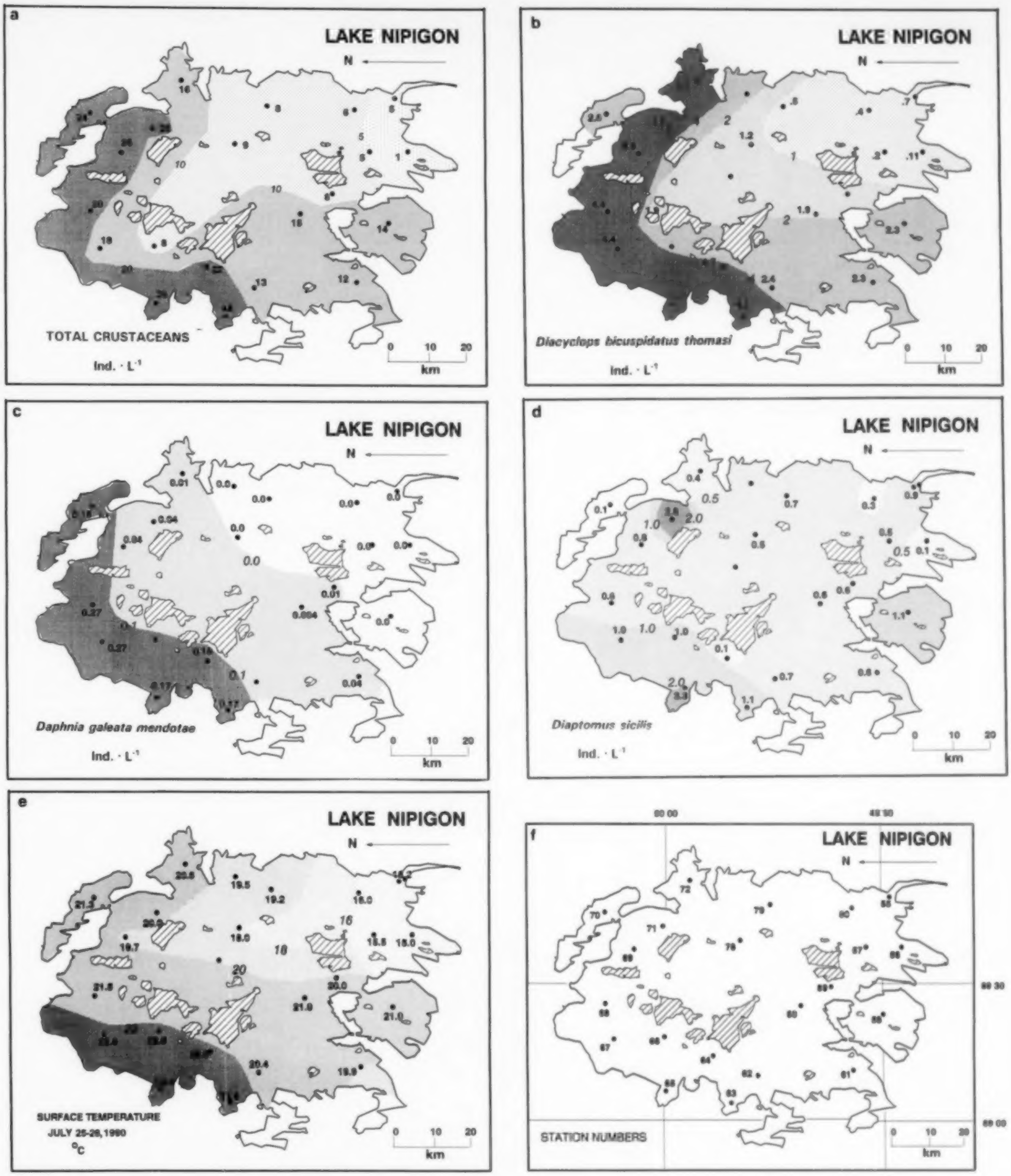
a
LAKE NIPIGON
N
TOTAL CRUSTACEANS
Ind. · L⁻¹
0 10 20
km
b
LAKE NIPIGON
N
Diacyclops bicuspidatus thomasi
Ind. · L⁻¹
0 10 20
km
c
LAKE NIPIGON
N
Daphnia galeata mendotae
Ind. · L⁻¹
0 10 20
km
d
LAKE NIPIGON
N
Diaptomus sicilis
Ind. · L⁻¹
0 10 20
km
e
LAKE NIPIGON
N
SURFACE TEMPERATURE
JULY 25-26, 1990
°C
0 10 20
km
f
LAKE NIPIGON
N
STATION NUMBERS
0 10 20
km

FIG. 4. Spatial distributions in Lake Nipigon. (a) Total crustaceans; (b) *Diacyclops bicuspidatus thomasi*; (c) *Daphnia galeata mendotae*; (d) *Diaptomus sicilis*; (e) surface temperature; (f) sampling stations.

Abundance and Composition of Crustacean Species

The average numbers of planktonic crustaceans found within each lake did not exhibit any trend with increasing lake size (Fig. 6). The three highest values were recorded from Linge, Green, and Trout lakes and the four lowest from Musclow and Orange lakes and Lakes Nipigon and Superior. The mean values were between 12 and 36 ind. · L^{-1}, with lower and higher values

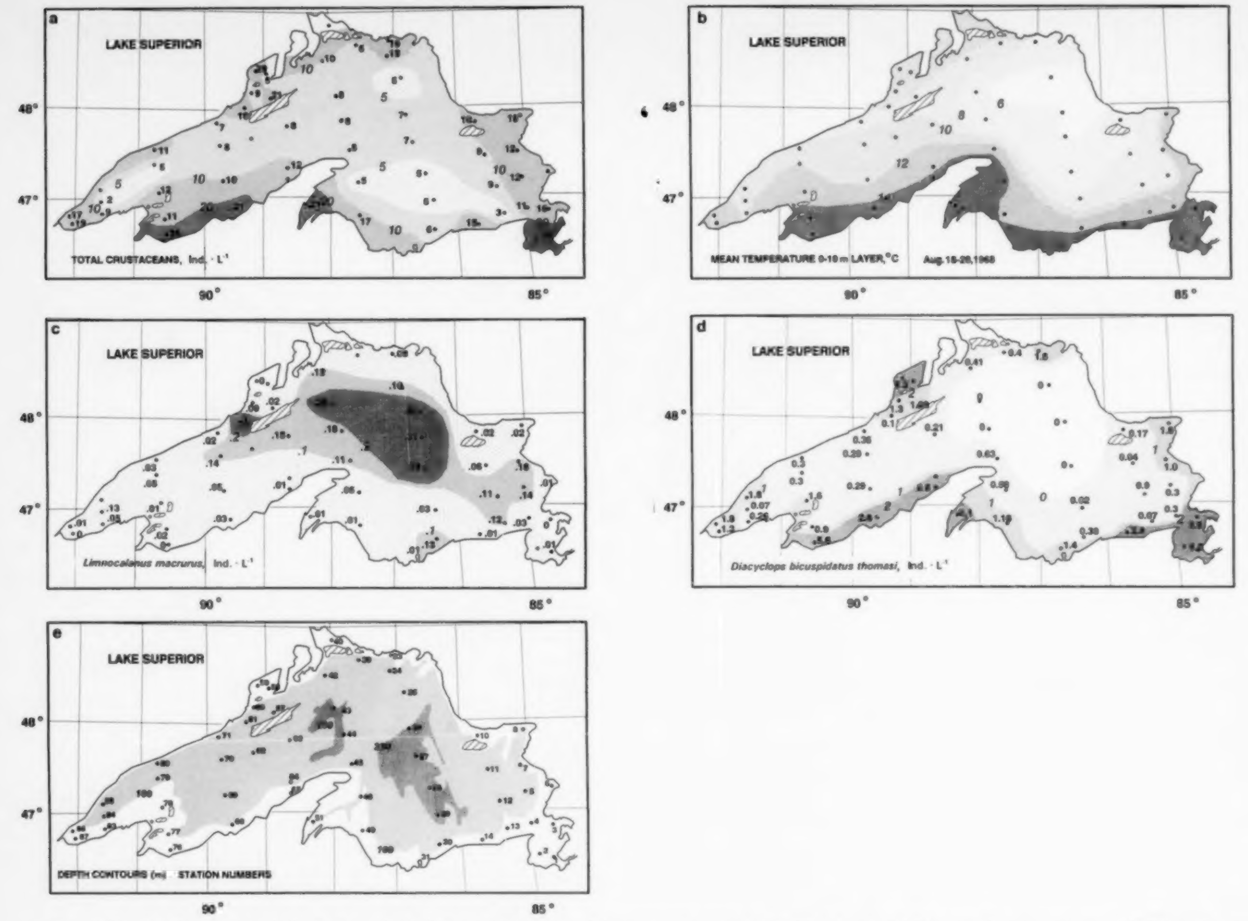
a
LAKE SUPERIOR
TOTAL CRUSTACEANS, Ind. · L^{-1}
b
LAKE SUPERIOR
MEAN TEMPERATURE 0-10 m LAYER, °C Aug. 18-26, 1968
c
LAKE SUPERIOR
Limnocalanus macrurus, Ind. · L^{-1}
d
LAKE SUPERIOR
Diacyclops bicuspidatus thomasi, Ind. · L^{-1}
e
LAKE SUPERIOR
DEPTH CONTOURS (m) STATION NUMBERS
48°
47°
90°
85°

FIG. 5. Spatial distributions in Lake Superior. (a) Total crustaceans; (b) mean temperatures, 0–10 m layer; (c) *Limnocalanus macrurus*; (d) *Diacyclops bicuspidatus thomasi*; (e) depth contours and sampling stations.

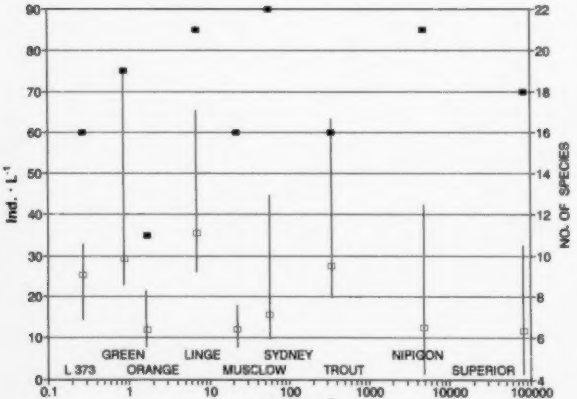

FIG. 6. Average (open squares) ranges (vertical lines) of total crustacean abundance and number of species (solid squares) in the study lakes.

found at either end of the lake size spectrum.

The well-documented trend of increasing structural complexity in fish communities with increasing lake size (Matuszek and Beggs 1988) suggested that a similar trend might be found in other biotic components. However, the pelagic crustacean communities in the study lakes (Table 2) did not conform to this relationship. From 11 to 22 species were found in small- to medium-sized lakes; no more than 18–21 species were present in samples from the two largest lakes, Nipigon and Superior (Fig. 6). However, a cluster analysis demonstrated that Lakes Nipigon and Superior were most similar and distinctly different from the remaining lakes (see Fig. 14a, 14b, and 15a) in spite of comparable numbers of species. Nine species were common to all nine lakes (*Epischura lacustris, Diaptomus minutus, Diacyclops bicuspidatus thomasi, Acanthocyclops vernalis, Daphnia galeata mendotae, Bosmina longirostris, Diaphanosoma leuchtenbergianum,* and *Leptodora kindtii*). The differences between Lakes Nipigon and Superior and the others are mainly due to the high numbers of calanoids species in the large lakes. Seven to eight calanoid species were found in Lakes Nipigon and Superior, while three to five species were usually encountered in the smaller lakes. *Senecella calanoides, Limnocalanus macrurus, Eurytemora affinis*, and *Diaptomus*

TABLE 2. Species composition of planktonic crustaceans in the study lakes. Species abundances are presented as percentages of the volume-weighted lake average.

Species	Lake								
	Lake 373	Green	Orange	Linge	Musclow	Sydney	Trout	Nipigon	Superior
Senecella calanoides Juday						0.14		0.02	0.05
Limnocalanus macrurus Sars			3.41		4.45	1.74	0.68	0.68	0.74
Eurytemora affinis (Poppe)									0.01
Epischura lacustris Forbes	0.73	0.37	1.74	0.03	0.75	0.54	0.54	0.28	0.02
Diaptomus minutus Lilljeborg	8.08	10.20	14.57	2.33	7.55	2.70	12.14	1.23	1.17
Diaptomus oregonensis Lilljeborg	0.02	0.04		2.27		6.31	3.38	0.06	0.19
Diaptomus sicilis Forbes	30.25	2.51		6.26	12.35	9.61		5.65	14.75
Diaptomus ashlandi Marsh								6.26	7.26
Diaptomus leptopus Forbes				0.0001		0.03			
Calanoida nauplii	1.35	1.12	1.44	9.38	2.73	10.84	16.04	14.79	33.22
Diacyclops bicuspidatus thomasi Forbes	45.38	14.88	26.87	12.42	0.03	11.33	19.57	17.42	10.27
Acanthocyclops vernalis Fischer	0.24	7.70	5.00	0.71	9.37	2.53	0.76	0.11	0.30
Mesocyclops edax (Forbes)	0.25	1.99		1.13	3.10	1.31	0.83	0.02	0.11
Tropocyclops prasinus mexicanus Kiefer	0.16	0.57	0.11	0.19	0.37	0.21		0.01	
Eucyclops speratus (Lilljeborg)		0.03		0.06	0.002				
Eucyclops agilis (Koch)				0.0003					
Cyclops varicans rubellus Lilljeborg				0.03					
Cyclopoida nauplii	3.02	19.66	20.93	35.72	48.65	21.80	30.72	44.93	22.09
Daphnia longiremis Sars	0.10	1.37					1.45	0.16	
Daphnia retrocurva Forbes	0.06	2.14		1.42		1.51	2.08	0.94	2.77
Daphnia galeata mendotae Birge	4.87	4.20	4.95	5.13	2.66	2.16	1.82	0.36	1.69
Daphnia schoedleri Sars		0.04			0.07	0.10			
Ceriodaphnia lacustris Birge	0.19			1.37		0.01			
Chydorus sphaericus (Mueller)	0.02	18.10		6.36	1.90	9.12	2.31	0.001	
Bosmina longirostris (Mueller)	3.14	9.32	16.77	9.60	1.97	2.52	6.38	6.87	5.11
Holopedium gibberum Zaddach		0.75	1.92	0.76	0.001	1.15	0.29	0.08	0.17
Diaphanosoma leuchtenbergianum Fischer	2.17	4.96	2.29	4.83	4.05	14.42	0.98	0.09	0.02
Sida crystallina (Mueller)		0.0003		0.0002		0.01	0.001	0.001	
Leptodora kindtii (Focke)	0.01	0.02	0.01	0.004	0.001	0.01	0.01	0.03	0.05
Polyphemus pediculus (Linne)						0.001		0.03	0.01
Total abundance (ind. · L^{-1})	24.56	35.55	11.86	35.65	12.00	15.64	28.00	15.26	11.71
No. of species	16	19	11	21	16	22	16	21	18

ashlandi were usually found in the larger lakes. On the other hand, a low proportion of cladocerans (8.5–9.8%) distinguished the two large lakes from all the others in which cladocerans accounted for 23% (10.6–40.9%) of the community composition.

A Measure of Plankton Homogeneity within Lakes

Similarity indices between pairs of stations were averaged in order to compute an integrated measure of the homogeneity of plankton within each lake and to compare these values among lakes. From 36 to 300 indices were averaged for lakes with 9–50 stations, respectively. Both Jaccard's (Fig. 7) and Renkonen's (Fig. 8) indices were applied. The 0.95 confidence intervals were relatively similar in all lakes and ranged from ±0.015 to 0.032 (mean 0.023) for Jaccard's index and from ±0.010 to 0.067 (mean 0.033) for Renkonen's index.

According to Jaccard's index, the lakes can be subdivided into two groups. The highest similarities (0.78–0.91) were found in the five small- to midsized lakes (Green, Orange, Linge, Musclow, and Sydney). In this group, there was no relationship between the level of similarity and either lake size or interstation distance. The lowest similarities (0.60–0.74) were encountered in the three largest lakes (Trout, Nipigon, and Superior) and in the smallest lake (Lake 373). This indicates that lake size or interstation distances per se do not greatly influence the homogeneity of zooplankton, as the lowest similarities were found at both ends of the size spectrum.

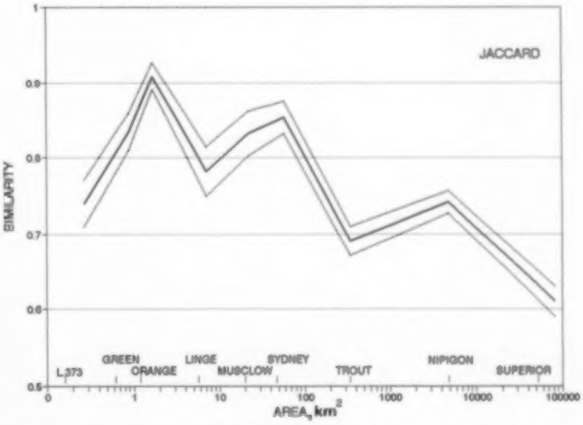

FIG. 7. Average Jaccard's similarity among stations within lakes. The solid line indicates the average similarity and the dotted lines the 0.95 confidence limit.

While Jaccard's community index emphasizes the presence or absence of species, Renkonen's percentage similarity placed

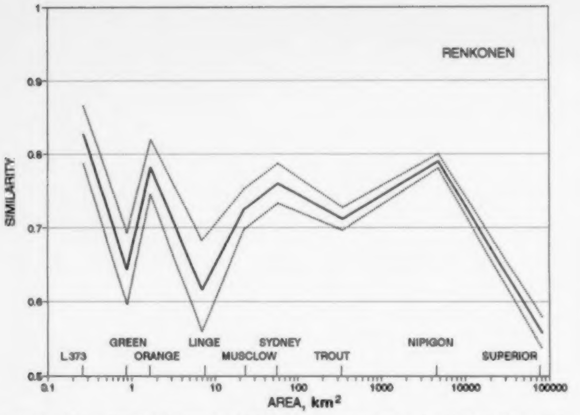

FIG. 8. Average Renkonen's similarity among stations within lakes. The solid line indicates the average similarity and the dotted lines the 0.95 confidence limit.

more weight on abundant species (Fig. 8). Similarities were about 10% lower, on average, than Jaccard's values. In other words, although the species were similar, their proportions at each station differed. The average Renkonen's similarity among stations ranged from 0.56 to 0.82, compared with Jaccard's range of 0.62–0.92. Renkonen's similarity did not exhibit any trend with lake size although Lake Superior had the lowest similarity among stations.

In addition to the above average measure of similarity among stations, a method of constrained clustering which allows only neighbouring stations to cluster (Legendre and Legendre 1984) was applied to elucidate the geographic aspect of plankton homogeneity. Maps were produced for selected lakes representing the smallest, medium, and largest sizes (Fig. 9, 10, and 11). The stations were clustered at various levels of similarity: 0.6–0.7, 0.7–0.8, and 0.8–0.9, and >0.9 (where applicable). The clusters were described with different densities of shading. Each shaded area included only stations with the indicated range of similarity and represented plankton patches of various sizes and shapes.

In Lake 373 (Fig. 9a), a patch of Jaccard's similarity, above 0.8, included three stations and encompassed an area about 200 × 300 m. With resolution relaxed to 0.7 similarity, most of the open-water area could be considered as a patch extending over 500 m. Similar patterns emerged from the maps based on Renkonen's similarity (Fig. 9b). The central stations surrounding the area of maximum depth (Fig. 3e) exhibited a very homogenous plankton community, with a similarity level >0.9. Most of the lake area shows similarity >0.8, and only one station in the northeastern bay differed (similarity of 0.6).

Musclow Lake plankton (Fig. 10a) was very homogenous. Most of the open-water stations displayed similarities above the 0.8 level of Jaccard's index, with the patch extending over 7 km. Slightly lower similarity (0.7–0.8) was observed between the open-water and inshore stations. Renkonen's similarity map (Fig. 10b) produced similar patterns, with more similar plankton in the group of stations near the center of the lake than near the shore.

In Lake Superior, patches of very similar plankton (Jaccard's index >0.8) occurred in the central-northern part of the lake and extended over an area 200 km long (Fig. 11a). With the definition of "similar plankton" relaxed to >0.7, the size of a patch extends to 300 km. Most of the areas with plankton of similarity >0.7 are located in the deepest part of the lake. South of these areas, a zone existed with similarities <0.6, indicating much larger differences between the plankton at the central and southern inshore stations. Along the southern shore, plankton was not as homogenous as in the central-northern part, and similarities between neighbouring stations often dropped below the 0.6 level. However, within these southern inshore areas, patches existed where two or three neighbouring stations located 30–50 km from each other had highly similar plankton (>0.8). Similar patterns could be observed on the maps based on Renkonen's similarity index (Fig. 11b). The most similar plankton was found in the deepest area of the lake (Fig. 5e). Stations with highly similar plankton (Renkonen's index >0.9) formed patches from 100 to 200 km long and up to 300 km with the definition relaxed to >0.7. The plankton at stations along the southern shore differed substantially both from that in the open-water area and from each other. In some areas, however, patches larger than 50 km existed with Renkonen's similarity above 0.7 or even 0.8. Those patches of relatively homogenous plankton consisted of different species as indicated in Fig. 5b, 5c, and 5d. These patterns confirmed the observations of Watson and Wilson (1978) who concluded that: . . . "By the time of July–August cruise, inshore clustering was very evident, as well as highly diverse, although the offshore region remained uniform in its relative species composition."

Number of Species Found as a Function of Sampling Intensity

By increasing the number of sampling stations, one would expect the cumulative number of species to increase until the total number of species present in the lake was found. To establish empirically the minimum number of stations required to obtain a complete list of species for each lake, it was first assumed that the number of stations established (9–50, see Table 1) provided an estimate of 100% of species present. Then, every second station was eliminated and species counted. The reduced numbers of stations were selected so that they provided the best possible coverage of the entire lake area. This procedure was repeated until a single station located near the centre of the lake remained (Fig. 12).

In Green and Orange lakes, between 95 and 100% of species were observed at the central station. In Musclow and Sydney lakes, from 69 to 72% of species were found at the single station. In Lake Superior, no more than 28% of species were found at that station. It therefore appears that the proportion of species detected at the single station is a function of lake size. There were, however, some exceptions to this pattern. In Lake 373, for example, in contrast with the other smaller lakes, only 56% of species were found at the central station. In this lake, more than 50% of the species occurred in very low numbers. Some were found only at a single station, with the result that catching all species in one sample was highly unlikely. However, a single station usually provided all the species that contributed more than 1% to the total abundance.

Figure 12 answers another question. How many stations had to be sampled to capture 90% of species? It appeared that one station was sufficient in Green and Orange lakes but as many as 15 stations were required in Lake Superior. These extreme lakes seem to confirm the trend toward a decreasing proportion of species per single station with increasing lake size, as described above, although not all the lakes between the extremes followed the same pattern. Particularly, in Lake 373, at least six stations were needed to catch 90% of species. This is not surprising, as the same lake also exhibited a very low average similarity among

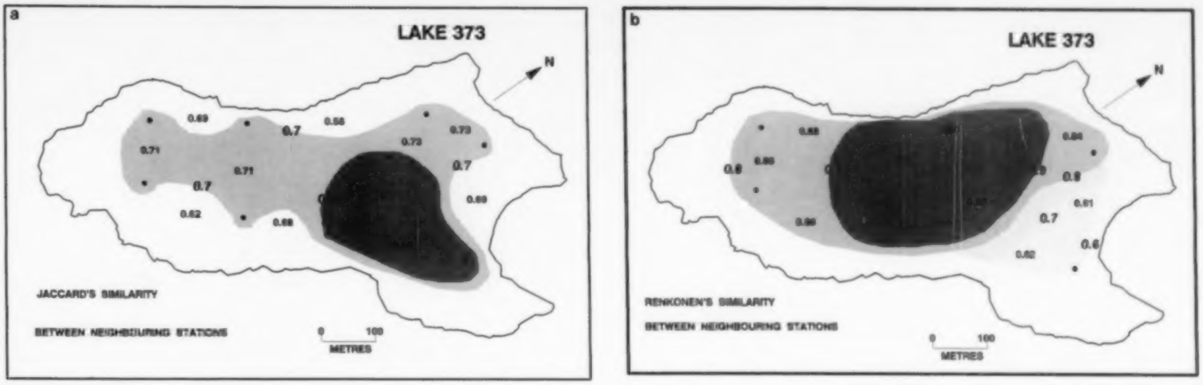
a
LAKE 373
N
JACCARD'S SIMILARITY
BETWEEN NEIGHBOURING STATIONS
0
100
METRES
b
LAKE 373
N
RENKONEN'S SIMILARITY
BETWEEN NEIGHBOURING STATIONS
0
100
METRES

FIG. 9. Similarity between neighbouring stations in Lake 373 (constrained clustering). Stations of the same range of similarity are clustered within the differentially shaded areas. (a) Jaccard's similarity; (b) Renkonen's similarity.

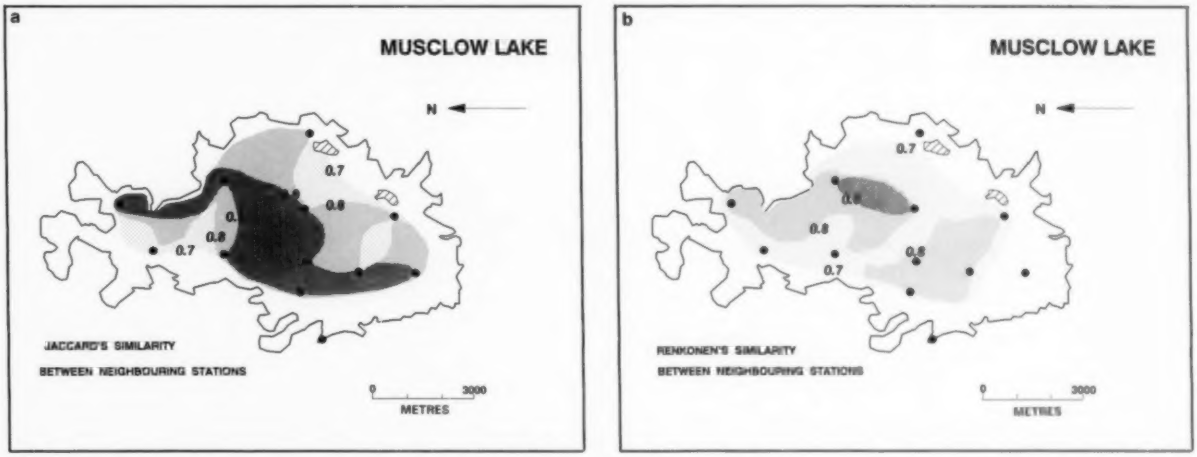
a
MUSCLOW LAKE
N
0.7
0.8
0.8
0.7
JACCARD'S SIMILARITY
BETWEEN NEIGHBOURING STATIONS
0
3000
METRES
b
MUSCLOW LAKE
N
0.7
0.8
0.8
0.7
RENKONEN'S SIMILARITY
BETWEEN NEIGHBOURING STATIONS
0
3000
METRES

FIG. 10. Similarity between neighbouring stations in Musclow Lake (constrained clustering). (a) Jaccard's similarity; (b) Renkonen's similarity.

stations (Fig. 7). The high level of heterogeneity found in this smallest lake is discussed below.

Average Abundance as a Function of Sampling Intensity

A similar procedure was applied to test empirically the effect of sampling intensity on estimates of total plankton abundance. It was assumed that the closest approximation to the actual crustacean abundance was given by the volume-weighted lake average (VWLA) calculated from the maximum number of stations in the lake. Averages based on declining numbers of stations are plotted as percentages of the VWLA in Fig. 13. The single, centrally located station estimated from 56 to 115% of VWLA plankton. The lowest values (56 and 71%) were from the two largest lakes, Nipigon and Superior; the highest values (107 to 115%) were from the three smallest lakes, Lake 373, Linge, and Orange. It appears that the larger the lake the more underestimated was the VWLA based on the central station. As the number of stations increased, the range of estimates narrowed. Sampling six stations, in most of the small- and medium-sized lakes, provided estimates that were within a range of ±10% of VWLA. Only in the two largest lakes did deviations from the VWLA remain higher, from ±20 to ±35% of the VWLA. At least 10 stations were necessary in these large lakes to narrow the range of estimates to ±10%.

How Well Does a Sample from a Single Central Station Represent the Plankton of the Entire Lake?

In circumstances where the researcher is restricted to a reduced number of samples, a centrally located station is usually selected. There is a common tendency to assume that such a station provides a reliable estimate of the plankton in a lake. One of the purposes of this paper was to test this assumption empirically. A partial answer was provided above (Fig. 12) with the demonstration that a single sample may capture from 28 (Lake Superior) to close to 100% of species (Green and Orange lakes). In addition, the larger the lake the more a single sample tends to underestimate the VWLA abundance of plankton (Fig. 13). These figures, however, do not indicate how accurately a single station sample represents the character of the planktonic community or how well it reflects the proportions of the species in a lake.

This question can be answered partially by comparing two dendrograms produced by multivariate similarity analyses: one based on the VWLA and the other based on the single sample

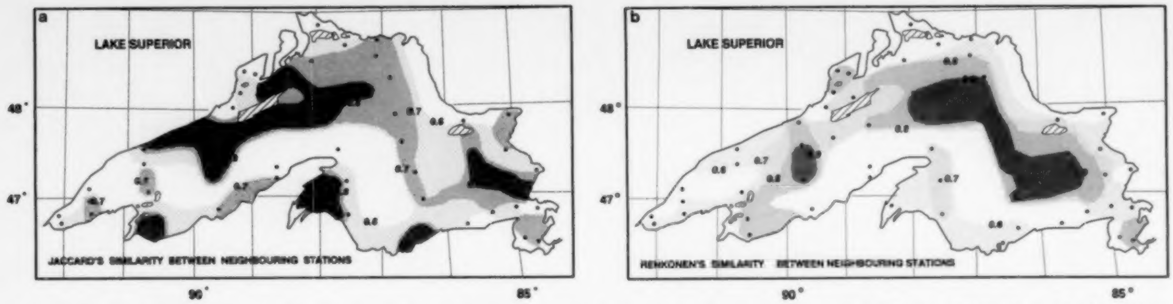
a
LAKE SUPERIOR
48°
47°
JACCARD'S SIMILARITY BETWEEN NEIGHBOURING STATIONS
90°
85°
b
LAKE SUPERIOR
48°
47°
RENKONEN'S SIMILARITY BETWEEN NEIGHBOURING STATIONS
90°
85°

FIG. 11. Similarity between neighbouring stations in Lake Superior (constrained clustering). (a) Jaccard's similarity; (b) Renkonen's similarity.

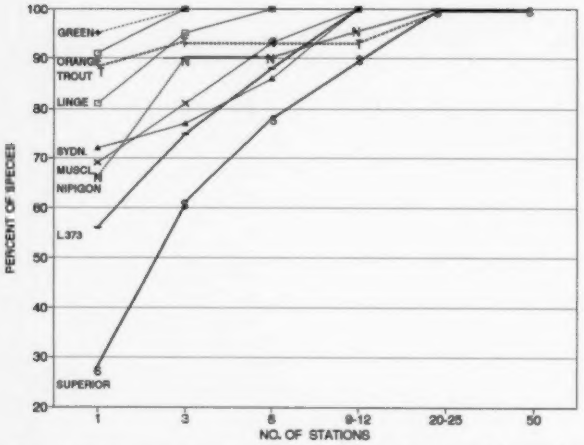

FIG. 12. Percentage of species encountered as a function of the number of stations in lakes of different size.

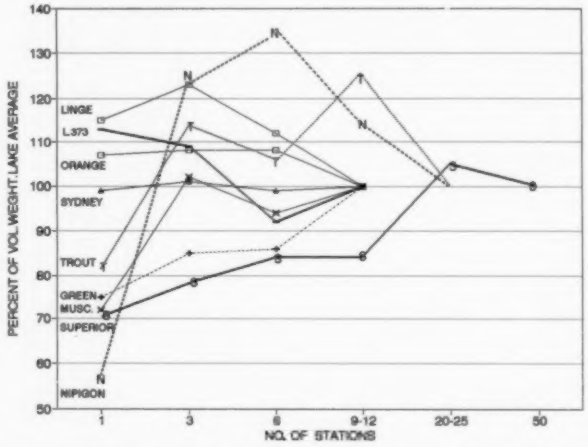

FIG. 13. Total crustacean abundance (percentage of the volume-weighted lake average abundance) as a function of the number of stations sampled in lakes of different size.

from the central station in each lake (Fig. 14a and 14b, respectively). Three major clusters can be recognized on the Jaccard dendrogram:

Lake average	Central station
Lake 373	
Green	Green
Linge	Linge
Sydney	Sydney
Trout	Trout
	Lake 373
Orange	Orange
Musclow	Musclow
Nipigon	Nipigon
Superior	Superior

Despite considerable variance among stations, as demonstrated previously, the clustering procedure resulted in similar groupings of lakes. Eight of nine lakes were classified in the same cluster in each dendrogram. Only smallest lake, Lake 373, was grouped differently in the two dendrograms, being closest to Green Lake in Fig. 14a (Lake average) but closest to Orange Lake in Fig. 14b (central station). The resemblance of these two Jaccard dendrograms indicated that the amount of information included in a sample from a single station is sufficient for a simple comparison of lakes based on the presence or absence of species. Although a single sample does not represent the plankton of the whole lake adequately, it captures sufficient qualitative aspects of the plankton to enable interlake comparisons.

The comparison of lakes based on Renkonen's index produced different results (Fig. 15a and 15b). The following clusters (with similarities >0.5) were recognized:

Lake average	Central station
Lake 373	
Orange	
Green	Green
Linge	Linge
Sydney	Nipigon
Musclow	
Trout	Orange
Nipigon	Sydney
Superior	Trout

Although Renkonen's index, like Jaccard's index, grouped lake averages into three clusters of increasing lake size, the dendrogram based on sampling single central stations was very different. Both smaller and larger lakes were found in each cluster, and only Green and Linge lakes remained close to each

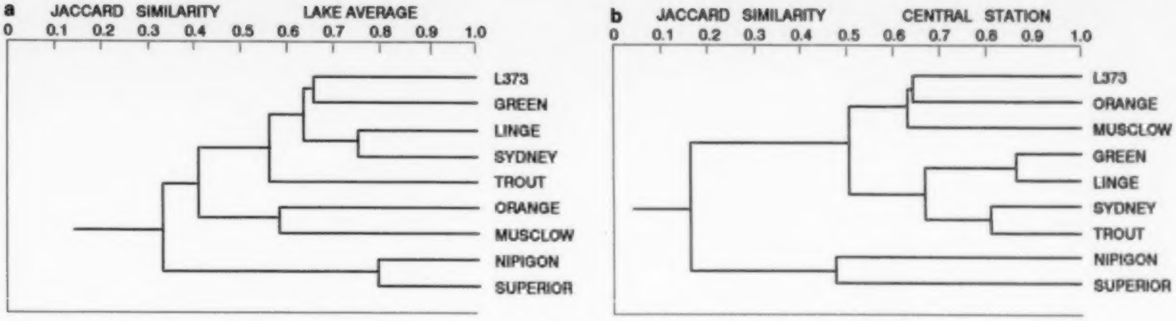

FIG. 14. Jaccard's similarity between (a) the volume-weighted lake averages and (b) the central stations in the nine study lakes.

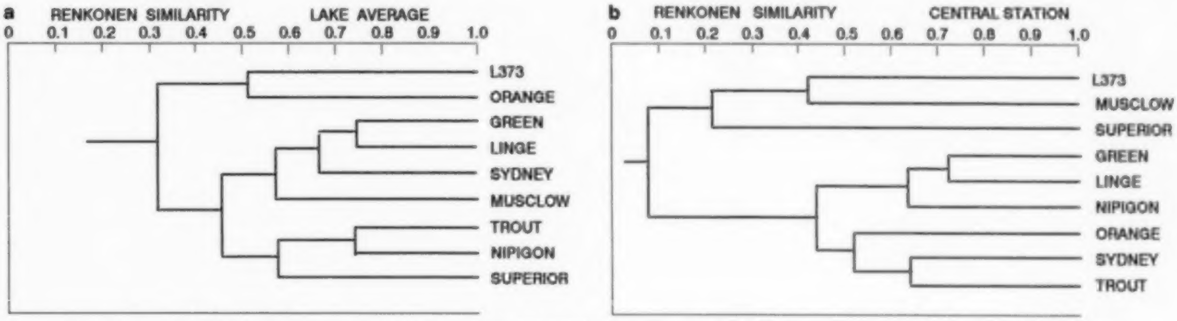

FIG. 15. Renkonen's similarity between (a) the volume-weighted lake averages and (b) the central stations in the nine study lakes.

other. Thus, it appears that a single sample from a lake has limited value for examining quantitative relationships among dominant species.

Another way of evaluating the accuracy of sampling a single central station for estimating the presence and proportions of individual species is to compare the abundance of a species at that station with that of the lake average. Such comparisons were produced for each lake, and selected examples representing various lake size categories are presented in Fig. 16. The VWLA total number of crustaceans was relatively accurately represented by the sample from the central station (for the nine lakes, 0.52–1.15 (mean 0.83) of the VWLA).

Individual species were less accurately represented by the central station sample, particularly in the larger lakes Nipigon (Fig. 16c) and Superior (Fig. 16d). In these lakes, most of the species (53–75%) were either absent at the central station or found in proportions either <0.25 or >1.75 of the VWLA. Not surprisingly, the central station of Lake Superior (Fig. 16d) was the least representative. It contained only 35% of the total number of species present.

In small- to medium-sized lakes (Fig. 16a and 16b) the central stations were more reliable in representing the VWLA. Sixty to 85% (mean 65%) of species were found within an interval of 0.25–1.75 of the VWLA and 22–58% (mean 45%) within 0.5–1.5 of the VWLA.

The results from Fig. 16 also provided some indication of trends in the spatial distribution of several species. *Diaptomus leptopus, Sida crystallina, Eucyclops agilis,* and *Cyclops varicans* were never found at the central station (marked as 0.00), as they usually either occurred in the inshore zone or were very rare. *Eucyclops speratus* and *Ceriodaphnia lacustris* were either absent or strongly underrepresented at the central station. On the other hand, *Diaptomus ashlandi, Diaptomus sicilis,* and *Limnocalanus macrurus* were often found in much higher numbers at the central station than in the other parts of the lakes.

In conclusion, in lakes of all sizes, a sample from a single central station represented the lake average total numbers of crustaceans relatively well; however, there was a tendency towards underestimation with increasing lake size. In small- to medium-sized lakes the sample from the central station underestimated the number of rare and littoral species but represented dominant species fairly well. With increasing lake size, the species composition of the planktonic community reflected by a single central station was more distorted. In the extreme case, as shown in Lake Superior, where two different types of communities (inshore and offshore) exist, the central station reflected only the offshore community.

Discussion

In the study lakes, no indication was found that species richness increases with lake size. This observation only apparently contradicts Dodson's (1992) findings. The highly significant correlation between log species number and log lake area ($r^2 = 0.57$) shown by Dodson was mainly due to inclusion of 23 (out of 66) very small water bodies (<1 ha in area) in his data set, while the smallest lake in our series, Lake 373, was 27 ha in area. There appeared to be only a very weak correlation in Dodson's species area curve in the set of lakes >1 ha. The larger lakes differed from the smaller and midsized ones not so much by their greater richness but mainly by their different composition of species. For example, some calanoid species such as *Senecella calanoides, Limnocalanus macrurus, Eurytemora affinis*, and *Diaptomus ashlandi* were found only in large and/or

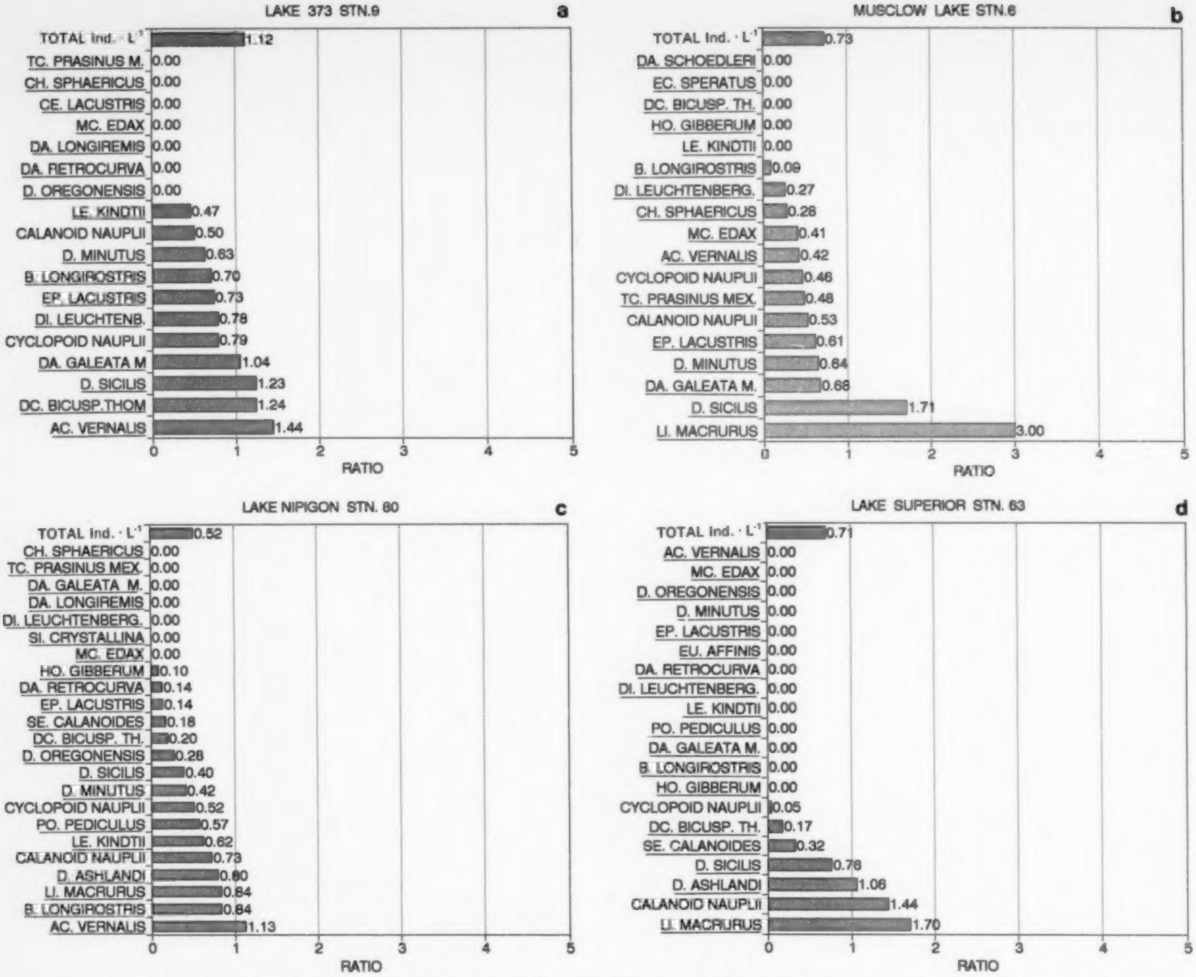

FIG. 16. Ratio of crustaceans found in the central station of the lake to the volume-weighted lake average. The value 0.00 denotes that a species, although present in the lake, was not found at the central station; values of 1.0 indicate that the central station contained numbers identical to the lake average. Species are arranged from under-represented to overrepresented. The greater the deviation of the bar length from 1.0 the larger the deformation of the central station sample from the lake average. (a) Lake 373; (b) Musclow Lake; (c) Lake Nipigon; (d) Lake Superior. Abbreviations: AC = *Acanthocyclops*, B = *Bosmina*, CH = *Chydorus*, C = *Cyclops*, CE = *Ceriodaphnia*, D = *Diaptomus*, DA = *Daphnia*, DC = *Diacyclops*, DI = *Diaphanosoma*, EC = *Eucyclops*, EP = *Epischura*, EU = *Eurytemora*, HO = *Holopedium*, LE = *Leptodora*, MC = *Mesocyclops*, PO = *Polyphemus*, SE = *Senecella*, SI = *Sida*, TC = *Tropocyclops*.

midsized lakes. On the other hand, *Diaptomus leptopus, Eucyclops speratus, Eucyclops agilis*, and *Daphnia schoedleri* were recorded in small- to midsized lakes.

Several general patterns emerged from the maps of distribution of individual species within each study lake (exemplified by Fig. 2, 3, 4, and 5):

(1) Smaller lakes (Lake 373, Green, Orange, and Musclow) were characterized by relatively uniform distributions of total crustaceans, with the highest densities rarely exceeding twice the lowest ones. Often, greatest abundances were found offshore, with numbers declining toward shore.

(2) With increasing size, lakes tended to develop much richer plankton communities in nearshore areas, often with densities several times higher than at midlake. Such a configuration seemed to be a function of the spatial temperature structure, with greater warming in shallower, inshore waters.

(3) While the spatial distributions of total numbers of crustaceans in smaller lakes appeared relatively homogeneous, individual species were dispersed much more heterogeneously.

(4) *Limnocalanus macrurus, Diaptomus sicilis*, and *Senecella calanoides* tended to occur in the central parts of both midsized and larger lakes. *Diacyclops bicuspidatus thomasi* usually inhabited offshore areas in smaller lakes but inshore areas in larger lakes.

(5) An increasing degree of insularity, shoreline development, and embayment creates conditions for increased heterogeneity in planktonic communities.

In an attempt to understand the spatial patterns described above the following explanation could be offered. Because the number of stations could not be increased proportionally with lake size, for practical reasons, interstation distances increased as a function of lake size. One would expect plankton similarity

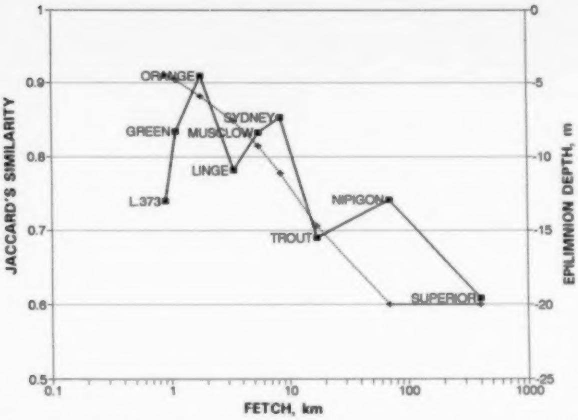

FIG. 17. Relationship between interstation similarity, fetch, and theoretical mixing depth in the nine study lakes. Solid squares = Jaccard's similarity among stations; crosses = theoretical mixing depth (Patalas 1984).

to decrease as interstation distance increases. This expectation was in part confirmed in Fig. 9, 10, and 11 which show closely located stations often clustered together.

As lake size increases, wind fetch increases and the tendency to deeper mixing increases (Arai 1981; Patalas 1984). Further, one can assume that the horizontal transport of water and wave height are also related to fetch. It has been shown that lakes with fetches of approximately 25 km reach midsummer asymptotic mixing depths of 15–20 m. No deeper mixing is observed even in lakes with fetches up to 400 km in length (Patalas 1984). Both Lakes Nipigon and Superior with fetches of 70 and 400 km, respectively, could be classified, according to the criteria proposed by Patalas (1990), in the category of large lakes.

Proceeding from Green to Sydney lakes (Fig. 17), the average similarity of plankton among stations was relatively constant (0.78–0.91). In these lakes, increasing interstation distance was compensated by increased horizontal turbulence. However, a significant difference was observed in Lakes Nipigon and Superior, which are of the size where isolated water masses can form and are not overcome by horizontal wind-related transport. The best evidence that such water masses occur in these lakes is the large variation in temperature at different stations. In Lake Nipigon, differences ranged from 15 to 23°C and in Lake Superior from 6 to 18°C (Fig. 4e and 5b). Trout Lake (fetch = 17 km), although below the critical fetch, exhibited lower similarity of plankton due mainly to extensive island development. This is probably the reason why Trout Lake clustered sometimes with the smaller lakes but at other times with Lakes Nipigon and Superior (Fig. 14a and 15b).

At the other end of the spectrum, the smallest lake in the series, Lake 373, also demonstrated a low mean similarity, 0.74, among stations, comparable with that in Trout Lake and Lakes Nipigon and Superior. In spite of the much shorter distances between stations (about 160 m), interstation similarity in Lake 373 was no higher than that in Lake Nipigon, with stations separated by distances of 15 km. This fact leads to the conclusion that mechanisms exist at both ends of the lake size spectrum that prevent efficient horizontal mixing. As shown above, in lakes with fetches >25 km, a further increase in lake size does not result in more intensive vertical and horizontal mixing (Patalas 1984) and the tendency to form isolated water masses increases. On the other hand, the very small lakes such as Lake 373 are well protected against wind by trees and surrounding hills. In such small lakes, the proportion of water surface protected by trees and hills, is much greater than in larger lakes. This conclusion can be supported by the comparable low similarities among stations in another group of lakes of similar size in the Experimental Lake Area (K. Patalas and A. Salki, in preparation).

Renkonen's similarity indices were usually lower than Jaccard's indicating that the proportions of species were more variable than their presence or absence. Renkonen's indices did not reveal the patterns described above. Higher and lower similarities (from 0.62 to 0.82, Fig. 8) were recorded in both the smaller and larger lakes. However, the lowest similarity (0.56) was still found in Lake Superior.

In conclusion, the patterns of plankton distribution vary with lake size. Smaller lakes require higher numbers of stations *per unit area* than larger lakes. Single central stations, although producing less precise estimates than lake volume-weighted average values, may provide for some purposes useful information, particularly for medium-sized lakes. The crucial point in plankton research is to maintain a balance between the amount of information a sampling method can provide and the conclusions drawn.

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Fish and Benthic Invertebrates: Community Concordance and Community–Environment Relationships

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Jackson, D.A., and H.H. Harvey. 1993. Fish and benthic invertebrates: community concordance and community–environment relationships. Can. J. Fish. Aquat. Sci. 50: 2641–2651.

Fish and benthic invertebrates from 40 lakes in south-central Ontario showed significantly concordant patterns based on community structure. Fish communities were associated significantly with lake morphological characteristics, but were uncorrelated with water chemistry. Large, deep lakes differed from shallow lakes in their fish species, having richer faunas due to the additional cold-water species. Centrarchid species occurred more frequently in small, shallow lakes than in larger lakes. The invertebrate community was not correlated with lake morphology, but showed a significant association with water chemistry, principally lake pH. A strong contrast in the abundance of *Chaoborus* and *Holopedium* existed, but it was unclear whether this was due to a predator–prey relationship or to differences in acid tolerance. Although the lakes showed similar patterns in the composition of both communities, each community was associated with a different set of environmental factors. Biotic processes within and between communities explain this paradox in community–environment relationships. Such biotic interactions may involve direct processes such as fish predation on a particular invertebrate taxon or indirect factors, e.g., where fish limit the abundance of invertebrate predators, thereby limiting the impact of these invertebrate predators.

On observe chez les populations de poissons et d'invertébrés benthiques de 40 lacs de la partie sud-centre de l'Ontario, des caractéristiques significativement concordantes qui relèvent de la structure des communautés. Il existe une association significative entre les communautés de poissons et les caractéristiques morphologiques des lacs, mais pas avec la chimie de l'eau. Les grands lacs profonds se distinguent des lacs peu profonds par leur composition spécifique; ils ont une faune plus riche du fait de la présence d'espèces d'eau froide. On observe les centrarchidés plus souvent dans les petits lacs peu profonds que dans les grands lacs. La communauté d'invertébrés n'est pas en corrélation avec la morphologie du lac, mais il existe une association significative avec la chimie de l'eau, principalement le pH du lac. Il existe un contraste marqué entre l'abondance de *Chaoborus* et de *Holopedium*; toutefois, on ignore si cette situation est attribuable à une relation prédateur–proie ou à des différences dans la tolérance à l'acidité. Même si la composition des deux communautés s'apparente d'un lac à l'autre, chacune de celle-ci est associée à un ensemble distinct de facteurs écologiques. Les mécanismes biotiques à l'oeuvre à l'intérieur des communautés et entre elles expliquent ce paradoxe observé dans les relations entre les communautés et le milieu. Ces interactions biotiques peuvent être constituées de mécanismes directs comme la prédation exercée par des poissons sur un taxon donné d'invertébrés, ou encore par des facteurs indirects comme la présence de poissons qui limitent l'abondance de prédateurs invertébrés, ce qui se trouve à limiter l'impact de ces derniers.

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Community ecologists typically study interspecific relationships or the relationship between a group of species and their physical–chemical environment. Such studies lead researchers to greater understanding of interspecific relationships, such as predation and competition, from which ideas can be formulated and tested experimentally. As well, relationships between communities and their environment provide strong evidence of the importance that various environmental factors have in determining species' distribution and abundance. Although recognition of the importance of community-level studies is increasing (e.g., Carpenter 1988a; Cole et al. 1991 and references therein), there are remarkably few studies examining communities of very different trophic or taxonomic level from the same environment. Within aquatic environments, numerous studies have considered fish, benthic invertebrate, phytoplankton, or zooplankton communities. Such research has concentrated on (1) a single community and the abiotic environment, (2) the effects that one or a few species from one community have on another community, (3) the changes in biomass and production across different communities, and (4) the changes in diversity of different communities due to environmental conditions.

Overall, ecologists generally have not considered whether different communities show similar patterns across varied environments and whether environmental conditions affect different communities in a comparable manner. For example, there is evidence that structure of fish communities is due to characteristics of lake morphometry (Harvey 1978, 1981; Tonn and Magnuson 1982; Jackson 1988), lake water chemistry (Harvey 1975; Somers and Harvey 1984; Rahel 1986; Kelso and

Lipsit 1988), and predation (Harvey 1981; Tonn and Magnuson 1982; Jackson 1988; Jackson et al. 1992). Similarly, researchers have concluded that zooplankton and benthic invertebrate communities are structured by fish predation (e.g., Brooks and Dodson 1965; Morin 1984; Post and Cucin 1984; Butler 1989; McQueen et al. 1990; but see Thorp and Bergey 1981a, 1981b), water chemistry (e.g., pH, phosphorus; Sprules 1975; Tessier and Horowitz 1990; Kilgour and Mackie 1991), and the complexity of the environment (Lodge et al. 1988).

More recently, experimental manipulation of lakes (e.g., Carpenter et al. 1987; Vanni 1988) has been used to study and test relationships between different taxocene or trophic levels while negating criticisms regarding enclosure/exclosure studies. Given the scope of these lake-wide manipulations and interlake variability, replication amongst lakes is impossible. In addition, results from such manipulations may differ when lakes have different environmental conditions (e.g., eutrophic versus oligotrophic or acid versus circumneutral). Comparisons of different communities across many lakes differing in environmental conditions require large, survey-based studies where the variation in different environmental conditions can be examined relative to the different communities.

Given the variation in importance attributed to different environmental factors in different systems having different species, our objective is to determine the relationship of fish and benthic invertebrate communities to their chemical and morphological environments. Specifically, we test whether lake water chemistry or morphology shows a greater association with the structure of both communities and whether both communities exhibit similar relationships with these abiotic components. Additionally, we test whether both communities exhibit similar patterns of lake association (i.e., do lakes show similar relationships in both community analyses?) across the lake set.

Methods

Data Collection

Forty lakes from south-central Ontario (see Table 1) were sampled for lake water chemistry, lake morphology, and the abundance of fish and benthic invertebrates. Lakes were chosen to provide equal representation across a range of surface area (9–124 ha) and pH (5.1–6.9). Chemical concentrations of calcium, magnesium, sodium, potassium, chloride, sulphate, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total phosphorus, and total nitrates were determined by the Ontario Ministry of the Environment (1981). Lake pH was determined in the field. Lake area, volume, maximum depth, and total shoreline perimeter were determined. Although compound or ratio variables (e.g., lake mean depth, lake area to watershed area) are often incorporated in such studies, they were not included due to their statistical properties and confounding natures (e.g., Kenney 1982; Jackson et al. 1990; Jackson and Somers 1991).

Fish species were sampled using fine- and coarse-meshed trap nets, baited minnow traps, clear plastic traps (Casselman and Harvey 1973), and multimeshed gill nets. Species abundance data from each sample were tabulated. Population estimates were calculated using mark–recapture methods in several lakes (Harvey and Lee 1981). Relative abundance estimates for each species from each sampling method were evaluated relative to Petersen population estimates. Despite intensive sampling (e.g., in excess of several hundred samples for some gears in some lakes), inconsistent rankings of abundance between gears within and across lakes for different species were found (D.A. Jackson, unpublished data). As the relative abundance data proved to be an unreliable indicator of abundance, analyses were restricted to fish species' presence–absence data. For community analyses, species found in only a single lake were excluded, as they contribute very little information in across-lake comparisons.

Benthic invertebrates were sampled by SCUBA divers. Sediment cores were taken at 10 locations along each of six transects in each lake (Allison and Harvey 1981; Collins et al. 1981). Transects were stratified relative to the proportion of each substrate type and stratum area following Allison and Harvey (1981). Each set of 10 cores was preserved immediately in 10% formalin. Invertebrates were separated from the substrate using a sucrose-floatation method (Allison and Harvey 1981) and generally identified to the family or order to remain consistent with previous studies (see Table 1) (Allison and Harvey 1981, 1988). These higher taxonomic levels have been recommended for marine community studies, as they retain the major trends present in studies using more resolved taxonomic levels, but reduce the large numbers of rare taxa (Warwick 1988, 1993). However, additional research is warranted as to the importance this taxonomic resolution has in the interpretation of freshwater community–environment patterns.

Nineteen of the 40 lakes were sampled in 1979 and 1980 for determination of chemistry, fish species, and benthic invertebrate abundance (Allison and Harvey 1981; Harvey and Lee 1981). The remaining 21 lakes were sampled in 1989 for determination of benthic invertebrates and water chemistry. The data for fish in these 21 lakes are from Jackson (1988). Additional details are found in Jackson (1992).

Statistical Analysis

Water chemistry and lake morphometry variables were transformed to approximate normal distributions and linearize bivariate relationships. Square-root transformations were used with potassium, sulphate, nitrate, phosphorus, lake surface area, volume, maximum depth, and perimeter. Magnesium concentration was arcsin-transformed and the remaining chemistry variables, except pH, were log-transformed.

Benthic data were first summarized within each lake. Various transformations of the benthic abundance data were made to linearize bivariate relationships and were based on preliminary analysis using Taylor's Power Law. However, the $\log_{10}(x + 1)$ transformation was found to provide results comparable with those using Taylor's Power Law. Similar findings have been indicated by Green (1979). Lake means were calculated using the transformed data and formed the basis of subsequent multivariate analyses.

Both the fish and invertebrate data sets were analyzed using correspondence analysis (CA). These communities were compared with the lake water chemistry and morphology data sets using canonical correspondence analysis (CCA, ter Braak 1986). This multivariate method summarizes the maximum amount of variation in one of the biotic data sets while constraining it to be associated with axes based on linear composites of the environmental data. For example, relationships among the invertebrate taxa are summarized such that the community relationships and the gradients in water chemistry are maximally correlated. The method permits a multivariate direct-gradient analysis of the community and environment.

Multivariate summaries of the benthic invertebrate and fish

TABLE 1. Codes for lake, fish, and invertebrate names.

Lake code	Lake	Fish code	Fish species	Invertebrate code	Invertebrate taxa
Bass	Basshaunt	B	Burbot (*Lota lota*)	Acar	Acari
Bent	Bentshoe	BBH	Brown bullhead (*Ictalurus nebulosus*)	Bosm	Bosminidae
Bigw	Bigwind	BM	Brassy minnow (*Hybognathus hankinsoni*)	Cala	Calanoida
B_Ch	Blue Chalk	BND	Blacknose dace (*Rhinichthys atratulus*)	Cera	Ceratopogonidae
Buch	Buchanan	BNM	Bluntnose minnow (*Pimephales notatus*)	Chaob	Chaoborinae
Cind	Cinder	BNS	Blacknose shiner (*Notropis heterolepis*)	Chir	Chironomidae
Clay	Clayton	BS	Brook stickleback (*Culaea inconstans*)	Chyd	Chydoridae
Cross	Crosson	BT	Brook trout (*Salvelinus fontinalis*)	Cycl	Cyclopoida
Dickie	Dickie	C	Cisco (*Coregonus artedi*)	Daph	Daphniidae
Gull	Gullfeather	CC	Creek chub (*Semotilus atromaculatus*)	Elmi	Elmidae
Harv	Harvey	CS	Common shiner (*Notropis cornutus*)	Ephe	Ephemeroptera
Heen	Heeney	FHM	Fathead minnow (*Pimephales promelas*)	Gast	Gastropoda
L_Cl	Little Clear	FSD	Finescale dace (*Phoxinus neogaeus*)	Harp	Harpacticoida
L_Fl	Lower Fletcher	GS	Golden shiner (*Notemigonus crysoleucas*)	Holo	Holopedidae
L_Wr	Little Wren	ID	Iowa darter (*Etheostoma exile*)	Macro	Macrothricidae
McKe	McKeown	LC	Lake chub (*Couesius plumbeus*)	Misc	Miscellaneous
Plas	Plastic	LMB	Largemouth bass (*Micropterus salmoides*)	Neur	Neuroptera
Poor	Poorhouse	LT	Lake trout (*Salvelinus namaycush*)	Nemt	Nemata
Porc	Porcupine	NRD	Northern redbelly dace (*Phoxinus eos*)	Odon	Odonata
R_Ch	Red Chalk	PD	Pearl dace (*Semotilus margarita*)	Olig	Oligochaeta
Rido	Ridout	PKS	Pumpkinseed (*Lepomis gibbosus*)	Ostr	Ostracoda
Soli	Solitaire	RB	Rock bass (*Ambloplites rupestris*)	Pele	Pelecypoda
Teap	Teapot	RT	Rainbow trout (*Oncorhynchus mykiss*)	Sidi	Sididae
Thre	Three Island	SMB	Smallmouth bass (*Micropterus dolomieu*)	Talt	Taltridae
Trou	Troutspawn	SS	Slimy sculpin (*Cottus cognatus*)	Tric	Trichoptera
Walk	Walker	WS	White sucker (*Catostomus commersoni*)		
		YP	Yellow perch (*Perca flavescens*)		

communities were compared using PROTEST (Jackson 1992). PROTEST is a test of matrix concordance incorporating Procrustean matrix rotation (Gower 1971, 1975; Digby and Kempton 1987; Rohlf 1990) where translation, rotation, reflection, and dilation fit one matrix to another matrix. In this example, the method tries to match the position of each lake in a multivariate space defined by the CA of the invertebrate data to the position of the same lake in the space defined by the CA of the fish species, thereby assessing the degree to which both communities have similar interlake patterns. The method

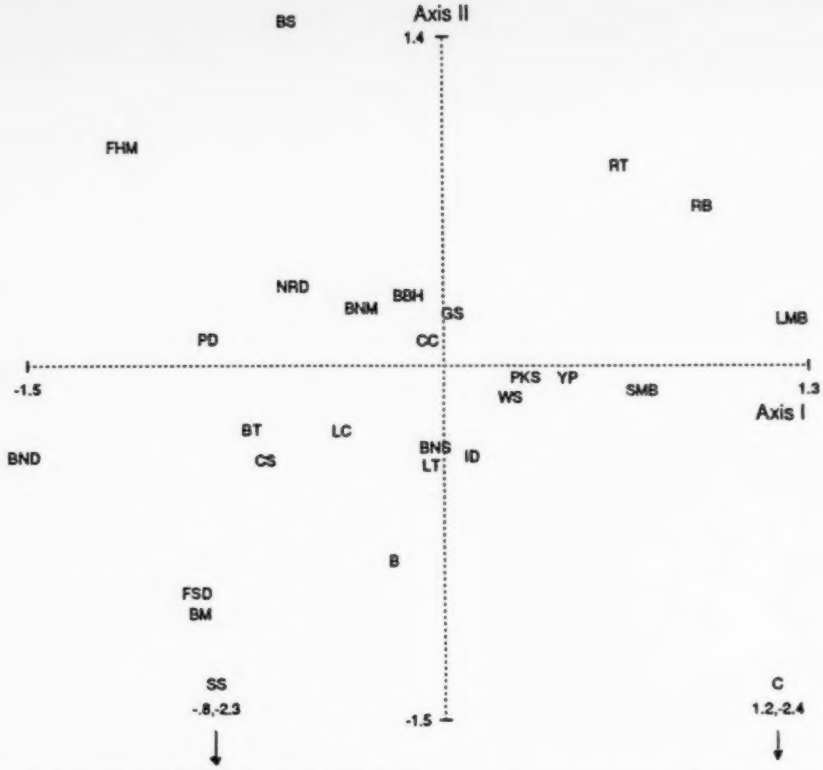

FIG. 1. Association of fish species from a CA of fish presence–absence across lakes. Species codes are defined in Table 1.

TABLE 2. Eigenvalues from principal components analysis (PCA) of lake water chemistry and morphology, CA of the benthic invertebrate and fish communities, and CCA of communities and environmental data.

	Eigenvalues				
Data set	Axis I	Axis II	Axis III	Axis IV	Total
PCA chemistry	4.879	2.396	1.497	1.153	12.00
PCA morphology	3.029	0.753	0.191	0.027	4.000
CA fish	0.272	0.217	0.181	0.179	2.032
CCA fish, chemistry	0.144	0.115	0.101	0.078	2.032
CCA fish, morphology	0.121	0.073	0.034	0.018	2.032
CA invertebrate	0.067	0.045	0.041	0.033	0.336
CCA invertebrate chemistry	0.042	0.019	0.017	0.012	0.336
CCA invertebrate, morphology	0.019	0.013	0.008	0.004	0.336

minimizes the sum-of-the-squared deviations (i.e., m^2; Gower 1975) between the pair of points representing each lake such that the greater the similarity of the multivariate configurations from the data sets, the lower the m^2 value. This measure is compared with that derived from repeatedly randomizing the configuration from one matrix and recalculating the m^2. The percentage of m^2 values equal to or less than the observed m^2 provides the significance level of the test.

Results

Fish Community–Environment Relationships

The CA of the fish community summarizes 42% of the total variance in the first four axes (Table 2). Plots of the species composition contrast small-bodied fishes, principally cyprinids, on the left side of the first axis with larger species, predominantly

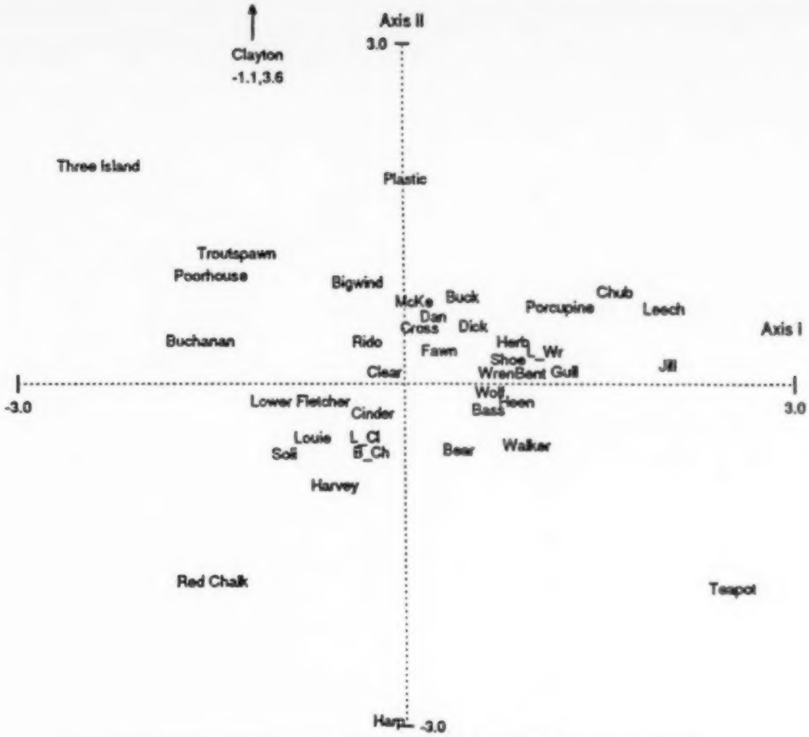

FIG. 2. Association of lakes based on a CA of fish species presence–absence.

centrarchids, which are positioned on the right (Fig. 1). No discernible pattern in composition was interpreted from the second axis. On the graph, a species is positioned at the centroid of the coordinates from the corresponding plot of lakes (i.e., Fig. 2) that contain the species. For example, fathead minnow occurs in Three Island, Troutspawn, Poorhouse, Solitaire, Bigwind, Buchanan, and Poorhouse lakes. Therefore, it is plotted at the centroid of the CA coordinates for these lakes. If one considers a line passing through the origin, then species located at the same end of the line are found together frequently whereas species located at opposite ends are found together rarely, if at all. Similarly, lakes positioned at opposite ends contain distinctly different fauna. For example, opposite the fathead minnow is the cisco which occurs in Teapot and Harp lakes. Fathead minnow and cisco do not co-occur in this lake set, and the lakes containing fathead minnow have a different fish fauna from those lakes containing cisco. Rarely occurring species (e.g., cisco, slimy sculpin) are positioned farther away from the graph origin, as are the lakes containing these rare species (e.g., Teapot and Harp).

The first axis from the CCA of the fish community and water chemistry was nonsignificant ($P = 0.50$). Therefore, no additional examination of this relationship is presented here, but additional details are found in Jackson (1992).

A CCA of fish species and lake morphometry shows a strong relationship between species such as lake trout, rainbow trout, cisco, burbot, and slimy sculpin and large, deep lakes (Fig. 3). Smaller, shallower lakes tended to include largemouth bass, rock bass, and brown bullhead. Several species, including smallmouth bass, golden shiner, pumpkinseed, yellow perch, white sucker, creek chub, northern redbelly dace, and Iowa darter, exhibit equal tendencies to occur in small and shallow or large and deep lakes.

Most lakes are easily categorized along the axis of depth and lake size (Fig. 4). Red Chalk, Harp, Blue Chalk, Clear, Bigwind, and Louie lakes are the larger, deeper lakes. Cinder and Bear lakes are somewhat atypical in that they are large, deep lakes, but they have extremely long lake perimeters relative to other lakes. The small, shallow, polymictic lakes include Leech, Jill, Dickie, and Fawn. Due to their relative shallow depths, these lakes typically do not stratify and have isothermal profiles during the late summer. Plastic Lake is positioned with this group but is deep enough to stratify thermally. Many lakes (e.g., Herb and Solitaire) are positioned orthogonal to the general lake size and depth axis and do not show similar trends in lake morphometry and fish species composition. The first two canonical axes account for 49 and 30% of the explained joint variance in fish species and lake morphometry. A significant relationship ($P = 0.03$) existed between the canonical axes of fish species composition and lake morphometry.

Invertebrate Community–Environment Relationships

The first four axes from a CA of benthic invertebrate abundance accounted for 55% of the total variance (Table 2). The first axis (20%) is dominated by the separation of *Chaoborus* from the remaining taxa. *Chaoborus* is positioned to the extreme right whereas several species (e.g., *Holopedium*) are located at the left end (Fig. 5). Increased occurrence and abundance of *Chaoborus* are associated with decreased occurrence and abundance of *Holopedium*, Pelecypoda, and Daphniidae. As *Chaoborus* and Bosminidae are positioned at right angles to one

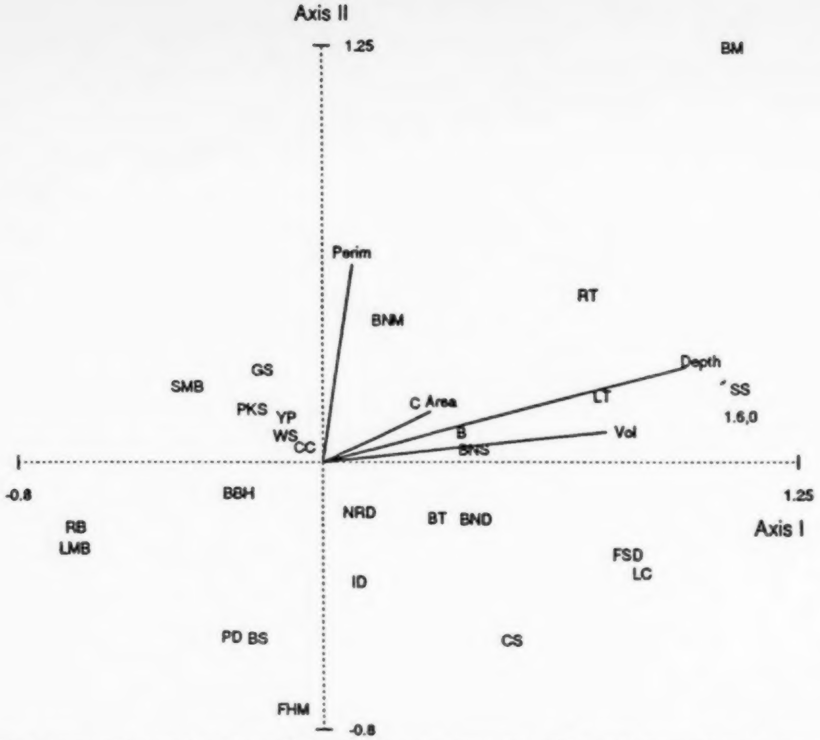

FIG. 3. Association of fish species and lake morphometry from a CCA of fish and lake morphometry across lakes.

another, the occurrence and abundance of these two taxa appear to vary independently of one another. In general, the second axis (13.5%) contrasts Bosminidae with the remaining taxa, particularly the Odonata, Macrothricidae, and Taltridae.

The plot of the lake ordination (Fig. 6) shows Fawn Lake at the extreme right end, followed by Dan, Poorhouse, and McKeown lakes. Overall, the only distinguishing trend along this axis is an increasing predominance of dystrophic lakes as one moves from left to right along the first axis. There are no apparent trends in the relative positioning of lakes along the second axis.

The chemistry-constrained ordination of the invertebrates led to a representation of the taxa similar to that found in the ordination of the invertebrates alone (Fig. 7). The first axis retains the contrast between *Chaoborus* and *Holopedium* whereas the second axis shows extremes of Bosminidae to Odonata. The first axis approximates a gradient of pH, calcium, and DOC, with the position of *Chaoborus* indicating a greater abundance at lower pH and lower calcium concentrations but higher DOC. The second axis shows a weak gradient associated with phosphorus and nitrates positively associated with Bosminidae and negatively with Ephemeroptera, Neuroptera, and Trichoptera.

The lake ordination has Fawn and Cinder lakes distinct from the remainder of the lakes (Fig. 8). Most lakes are clustered together in the central portion of the graph. When lake water chemistry is considered with the invertebrates, a pH gradient is found to extend from high-pH lakes (e.g., Blue Chalk and Red Chalk) to lakes of lower pH (e.g., Fawn and McKeown). The canonical axis, dominated by the pH gradient, is associated significantly ($P < 0.01$) with the first axis from the benthic invertebrates. This axis explains 34% of the joint variation in the invertebrate and chemistry data, indicating close correspondence between the invertebrate biota and their chemical environment.

The first axis from the CCA of the invertebrate community and lake morphology was nonsignificant ($P = 0.17$). Therefore, no additional examination of this relationship is presented here, but additional information can be found in Jackson (1992).

Comparison of Fish–Invertebrate Communities

Four CA axes from each of the fish and invertebrate ordinations were used in the PROTEST analysis. These axes were derived as weighted mean species scores so that the scaling represented an Euclidean distance plot. The test showed a significant concordance between the two matrices ($P = 0.02$), indicating that the arrangements of lakes in each ordination space are more similar than expected due to random chance. The inclusion of additional CA axes resulted in similar conclusions, but fewer axes yielded probabilities greater than 0.10.

Discussion

The fact that the lakes exhibit similar patterns based on their fish and benthic invertebrate communities may not be surprising. Considerable research has shown that fish can alter both the benthic and nektonic communities. Many studies have been based on enclosures where fish are held or exclosures where fish

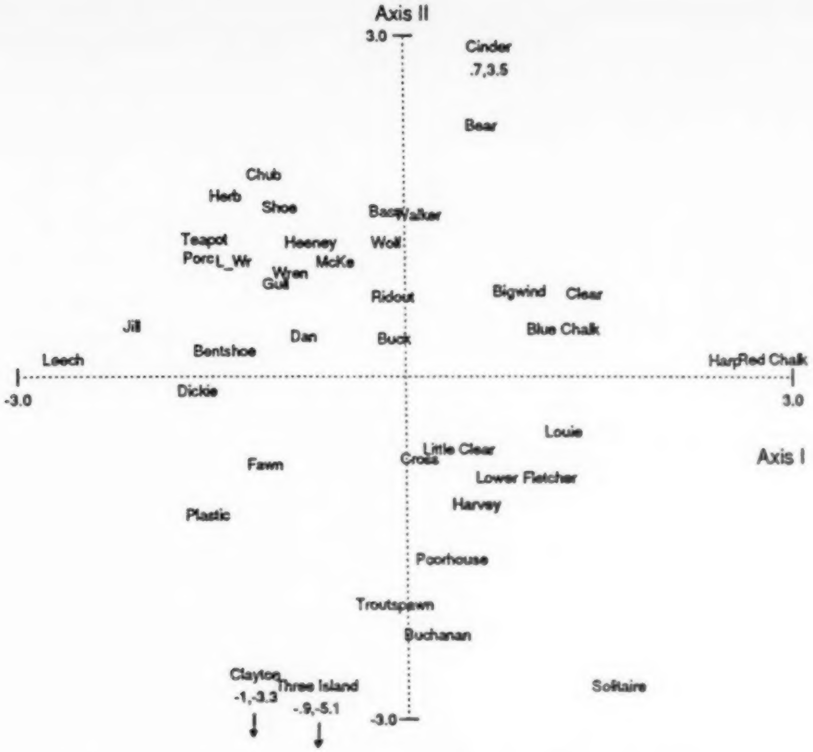

FIG. 4. Association of lakes based on a CCA of fish species and lake morphometry.

are excluded, but the treatment is assumed to have no direct affect on the benthic community. Several of these cage and whole-lake studies have documented changes in the composition of the invertebrates (Zaret and Paine 1973; Mills and Schiavone 1982; Magnan 1988; Butler 1989), reductions in the average size of the invertebrates (Morin 1984; Post and Cucin 1984), or reductions in the standing biomass but increased production of invertebrates (Post and Cucin 1984). There are other studies demonstrating a lack of effect (e.g., Thorp and Bergey 1981a). Results from enclosure/exclosure studies have been questioned regarding methodological problems (e.g., biofouling; Thorp and Bergey 1981a). As well, the spatial and temporal scale of these studies has been criticized, as ecological processes may differ at larger scales due to environmental heterogeneity and prey refuge, and seasonal effects may differ from those found in short-term enclosure experiments (Carpenter 1988b; Stein et al. 1988; Wellborn and Robinson 1991).

Rather paradoxically, our study shows similar structure between the fish and benthic invertebrate communities across the lakes, but different relationships for each taxonomic community and the lake environmental conditions. Fish communities appear to be structured more by the morphological characteristics of the lakes whereas the invertebrate results indicate that the chemical environment is more critical. The importance of lake morphology, particularly depth, to fish communities is well recognized (Johnson et al. 1977; Harvey 1978; Tonn and Magnuson 1982; Jackson 1988). Many species require cold-water refuges during summer; unless lakes are sufficiently deep and productivity too low to cause anaerobic conditions below the epilimnion, these species are excluded from the community. At the latitude and elevation of the lakes in this study, summer epilimnial temperatures may exceed the upper lethal temperatures of cold-water species (trout, char, and ciscoes) and approach those of cool-water species (e.g., white sucker).

During winter, low oxygen levels may develop under the ice cover of shallow lakes especially. Fish species differ in their tolerance to, and ability to find refuges from, such low-oxygen conditions. Species such as centrarchids and esocids show limited tolerance relative to cyprinid and ictalurid species. Thus, there may be community-wide selection of species based on their oxygen requirements (Jackson and Harvey 1989). In this study, the centrarchids were found to be associated with the smaller, shallower lakes (Fig. 7). In general, centrarchids are intolerant of low oxygen levels. Therefore, it can be inferred that winter oxygen conditions do not appear to be of major importance across all the lakes, but low levels may be important in specific lakes, e.g., Three Island (Jackson 1988). The environmental condition contributing the most to fish community structure is likely lake depth and the associated thermal stratification permitting survival of cold-water species such as lake trout, rainbow trout, and cisco during the summer. Thus, depth and the related temperatures and oxygen levels provide selective forces acting on the community throughout much of the year (Jackson et al. 1992).

The effect of water chemistry was inferred with the benthic invertebrate communities where a significant relationship was found between community composition and a pH-dominated environmental gradient. Several studies have shown a reduction

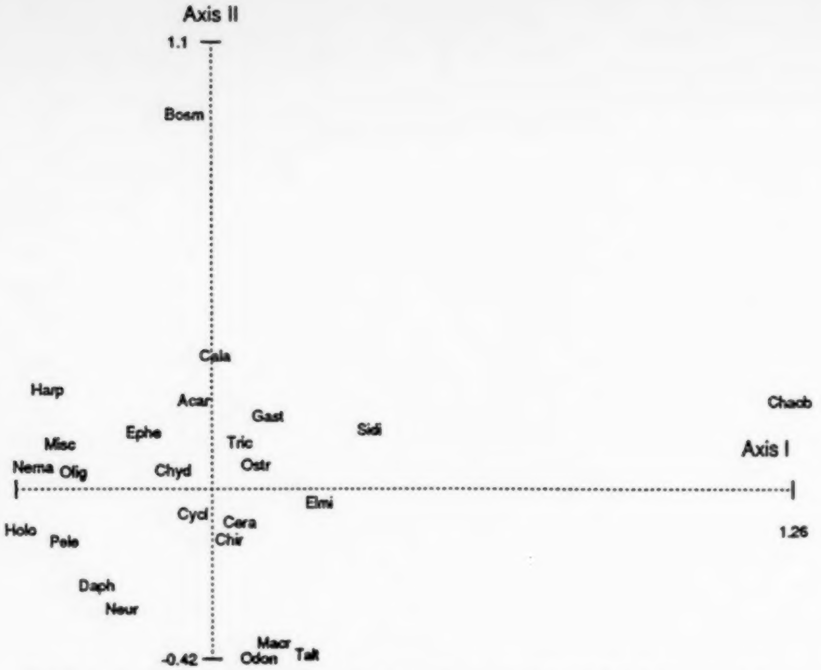

FIG. 5. Association of benthic invertebrates from a CA of invertebrate abundance across lakes.

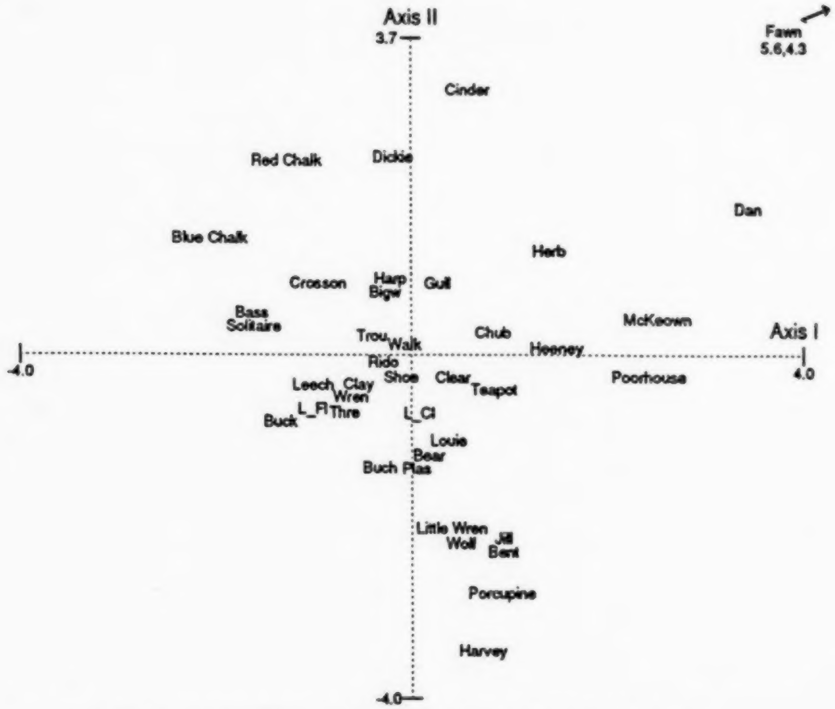

FIG. 6. Association of lakes based on a CA of benthic invertebrate abundance.

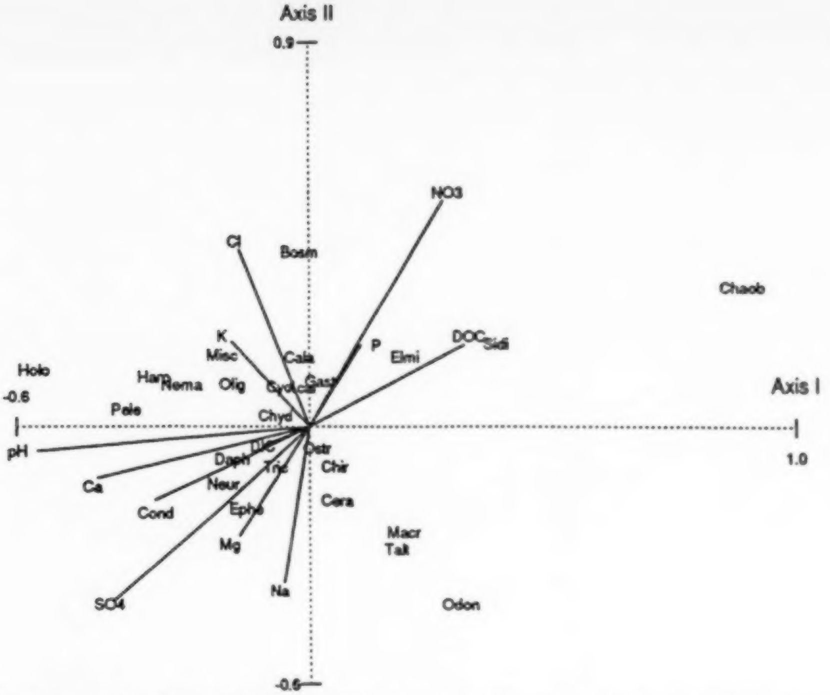

FIG. 7. Association of benthic invertebrates and water chemistry from a CCA of invertebrate abundance and water chemistry across lakes.

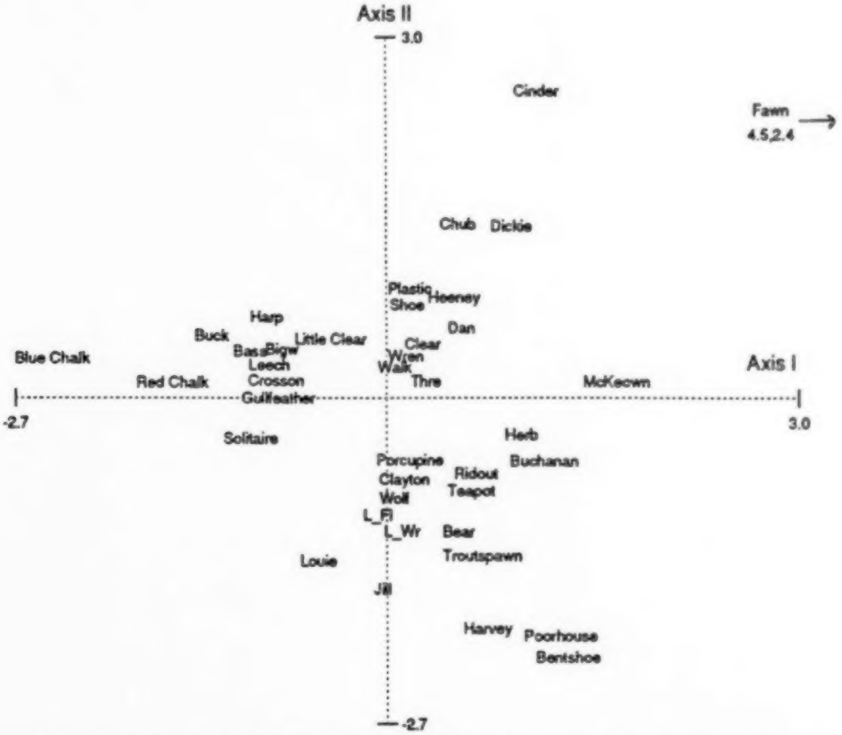

FIG. 8. Association of lakes based on a CCA of invertebrate species and lake water chemistry.

in taxonomic richness in acidified lakes (e.g., gastropods and pelecypods (Hagen and Langeland 1973; Roff and Kwiatkowski 1977; Collins et al. 1981; Harvey and McArdle 1986), macrothricids (Økland 1969; Harvey and McArdle 1986), *Mysis relicta* (Nero and Schindler 1983), and decapods (France 1984)). Reductions in the biomass or density of ephemeropterans, odonates, trichopterans, neuropterans, and plecopterans have all been found in acid-stressed lakes (Hagen and Langeland 1973; Grahn et al. 1974; Hendrey et al. 1976), although Harvey and McArdle (1986) found no reductions in these taxa.

Given the considerable variation and conflicting results among studies, the relationships between invertebrates and pH are unclear. Some of this variability may be attributed to the concentration and bioavailability of toxic metals, most especially aluminum (Stokes et al. 1989). Simultaneous changes occurring at different trophic levels within the lakes may also confound the relationship. Taxa such as fish and crayfish may limit the abundance of various benthic organisms through predation. As fish, crayfish, and additional predators are often intolerant of acid conditions, these species may be reduced or eliminated, thereby reducing predation pressure on more acid-tolerant taxa, and their numbers of prey may increase. This indirect effect of lake acidification has the potential to alter considerably the dynamics of ecosystems. Reductions in fish predation may lead to increased predation by *Chaoborus*, thereby altering the cladocerans and, subsequently, additional zooplankton and phytoplankton species via competition. Clearly, the complexity of these interactions complicates any direct assessment of the relationship between pH and the benthic community. Although the literature generally agrees that changes in the benthos occur, it is likely that some of the changes may be indirect rather than strictly due to the toxicity of the hydrogen ion per se.

Chaoborus abundance was associated with more acid lakes, but negatively correlated with *Holopedium*. Yan et al. (1985) found that *Chaoborus* abundance was greatest in acid lakes. Yan et al. (1988) showed a negative relationship between *Daphnia* spp. and *Holopedium*, but they could not determine whether differential predation or pH tolerance was more important in the negative correlation between the abundances of these taxa. Results show both the latter taxa to have similar occurrences and both are correlated negatively with *Chaoborus* abundance. Whether this pattern depends on a lesser tolerance to acid by Daphniidae and *Holopedium* relative to *Chaoborus* or a direct predator–prey effect between these taxa is unclear. The results show that lakes with circumneutral pH had increased abundances of *Holopedium*, Pelecypoda, Ephemeroptera, Neuroptera, Harpacticoida, and Nemata. Lakes with lower pH and higher DOC conditions had greater concentrations of *Chaoborus*, Sididae, and Elmidae relative to higher pH lakes. The most abundant taxon was Chironomidae which was ubiquitous and showed no trend in abundance or occurrence relative to lake acidity. Similar results were found by Harvey and McArdle (1986).

The paradox of a concordance between the fish and invertebrate communities but different relationships between the communities and the environment is due likely to an interaction between the biotic and abiotic environments. The fish community is structured primarily on the basis of the physical environment of the lake. Conditions necessary for spawning, e.g., shoals, as well as cold-water refuges for various species, may only be present in larger lakes. Within a general lake morphotype, chemical conditions such as pH and dissolved oxygen are likely important in determining the viability of certain species (e.g., fathead minnow and smallmouth bass). These particular factors contribute to determining the fish species that can survive within a lake. In addition to these factors, the abundance of fishes will be influenced by lake productivity via invertebrate productivity.

The invertebrate community is structured by the chemical portion of the abiotic environment. In conjunction with the influence of chemistry, the invertebrates are impacted by the fish community. The interactions between the fish and invertebrates are likely complex due to a variety of direct and indirect effects (sensu Kerfoot and Sih 1987). Some invertebrate taxa may be limited in abundance due to direct predation by fish or show increased abundance due to reduced invertebrate predation mediated by fish. Given the network of possible interactions between these two communities, it is difficult to predict the responses of many invertebrate taxa to changes in their chemical environment. The relationship of some invertebrate taxa (e.g. cladocerans) with different fish communities is predictable, and similar patterns result from surveys across lakes or from experimental manipulations. However, the response of many benthic taxa may be species specific as shown by changes in size structure of invertebrate communities (Post and Cucin 1984). A general shift to smaller taxa, reduced biomass, and increased rates of production is to be expected with increased predation by fish, but our understanding remains insufficient to permit more detailed predictions about benthic-community structure in response to changes in the fish community.

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Natural Hybridization between Arctic Char (*Salvelinus alpinus*) and Lake Trout (*S. namaycush*) in the Canadian Arctic

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Wilson, C.C., and P.D.N. Hebert. 1993. Natural hybridization between Arctic char (*Salvelinus alpinus*) and lake trout (*S. namaycush*) in the Canadian Arctic. Can. J. Fish. Aquat. Sci. 50: 2652–2658.

Natural interspecific hybrids between lake trout (*Salvelinus namaycush*) and Arctic char (*S. alpinus*) ranged in frequency from 1.8 to 6.8% in four of eleven lakes containing both species on the northern Melville Peninsula, N.W.T., and were also detected at several other sites across the Canadian Arctic. Hybrids were heterozygous for each of seven diagnostic allozyme loci between *S. alpinus* and *S. namaycush*, and were morphologically intermediate between the parental species. Restriction endonuclease analysis of mitochondrial DNA revealed that all but one of the hybrids had *S. namaycush* as their maternal parent. Resampling of two lakes where hybrids were detected revealed low levels of bidirectional nuclear introgression between the two species and limited transfer of *S. namaycush* mtDNA into *S. alpinus*.

Il y avait 1,8 à 6,8 % d'hybrides interspécifiques naturels du touladi (*Salvelinus namaycush*) et de l'Omble chevalier (*S. alpinus*) dans quatre de onze lacs où cohabitaient les deux espèces, dans la partie nord de la péninsule Melville, T.N.-O.; des hybrides ont été trouvés à plusieurs autres stations dans l'Arctique canadien. Chez ces hybrides, il y avait hétérozygotie d'allèles à chacun des sept locus d'allozymes diagnostiques, entre *S. alpinus* et *S. namaycush*; les hybrides étaient des intermédiaires morphologiques entre les espèces parentes. L'analyse par endonucléase de restriction de l'ADN mitochondrial a montré qu'à une exception près, la progénitrice des hybrides appartenait à l'espèce *S. namaycush*. Un nouvel échantillonnage dans deux lacs où des hybrides avaient été trouvés, a montré une introgression nucléaire bidirectionnelle à faible niveau entre les deux espèces ainsi qu'un transfert limité de l'ADNmt de *S. namaycush* à *S. alpinus*.

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Although interspecific hybridization has been abundantly demonstrated among fish species (Hubbs 1955; Schwartz 1972, 1981), very few studies have examined the directionality of natural hybridization events or the extent of genetic introgression which accompanies it. Neither morphological nor allozyme analyses provide the information necessary to ascertain the directionality of hybridization events (Verspoor and Hammar 1991). The recent development of mitochondrial DNA (mtDNA) techniques has provided a basis for the identification of the maternal parent of hybrids, but relatively few studies have made use of this technique. In a recent review, Billington and Hebert (1991) reported only 10 studies that used mtDNA to document the directionality of interspecific hybridization in natural fish populations. Together, these studies include four cases of unidirectional hybridization and six bidirectional crosses. In four of these cases, introgression between the parent species accompanied hybridization. None of these, however, involved studies of hybridization or introgression between natural populations of two native salmonid species.

Lake trout (*Salvelinus namaycush*) and Arctic char (*S. alpinus*) have broadly sympatric native distributions in North America, ranging from Alaska across the northern Yukon and central Northwest Territories to northern Quebec and Labrador (Scott and Crossman 1973). The two species also overlap in habitat utilization and diet (Johnson 1980; Martin and Olver 1980), although *S. namaycush* displaces *S. alpinus* as the top resident piscivore when the two species co-occur (Fraser and Power 1989). Morphological analyses (Behnke 1980) have suggested that *S. namaycush* and *S. alpinus* are sufficiently distinct to be placed in separate subgenera (*Cristivomer* and *Salvelinus*). Analyses of mitochondrial DNA divergence support this distinction, and suggest that *S. alpinus* and *S. namaycush* last shared a common ancestor approximately one million years ago (Grewe et al. 1990).

Although *S. alpinus* and *S. namaycush* are sympatric at many sites, there was until recently no evidence of natural hybridization between the species, or indeed between *S. namaycush* and any other *Salvelinus* species. Behnke (1980) linked the lack of hybrids to the taxonomic isolation of *S. namaycush*, and placed it as the sole member of the subgenus *Cristivomer*. Stewart and Bernier (1982, 1984) suspected hybridization between *S. alpinus* and *S. namaycush* based on pyloric caecae counts and body coloration, but genetic evidence was lacking until Hammar et al. (1989) reported the presence of two hybrid individuals in a northern Labrador lake. While their meristic and allozyme analyses made clear the hybrid nature of these fish, there remains a need to determine both if hybrids are more generally distributed and to ascertain if hybridization is accompanied by introgression. The present study examined the abundance of hybrids in lakes on the Melville Peninsula, Northwest Territories and used allozyme and mtDNA analyses to determine the directionality of hybridization and possible extent of introgression between the species.

Methods

Fish were collected from 12 lakes on the upper Melville Peninsula (Table 1) in August of 1989 and 1990 using 5- and 10-cm mesh gill nets at varying depths. Each fish was photographed and recorded for fork length, weight, sex, and reproductive state. Liver, kidney, and muscle samples were taken from each fish and kept on ice for 1–3 d, then frozen and stored at −80°C until analyzed. Heads were kept from fish from Ice Lake for ageing of otoliths. Supplementary collections from two lakes (Sarcpa and Lailor) where hybrids were detected were carried out in 1991. Muscle and adipose fin biopsies were taken for protein electrophoresis and mtDNA analyses, respectively. Muscle samples were immediately frozen and stored at −80°C. Fin samples were preserved in 70% ethanol and stored at 4°C.

Protein electrophoresis was carried out on cellulose acetate gels, using standard protocols (Hebert and Beaton 1989). Fish were screened for variation at several loci known to be polymorphic in lake trout (Dehring et al. 1981; Ihssen et al. 1988), as well as those loci used by Hammar et al. (1989) in their analysis of Labrador *S. alpinus* × *namaycush* hybrids (Table 2). Staining procedures followed those described in Hebert and Beaton (1989). The nomenclature conventions suggested by Shaklee et al. (1989) was used to describe the allozyme loci and allelic mobilities, assigning the most common Arctic char allele at each locus a value of 100.

Total DNA was extracted from frozen liver tissue and subjected to restriction fragment length polymorphism (RFLP) analysis following methods described in Sambrook et al. (1989). DNA from fin samples was extracted using the protocol of Taggart et al. (1991). The fish were screened for mitochondrial DNA (mtDNA) polymorphism with five restriction enzymes (*Ava*I, *Bam*HI, *Hind*III, *Pst*I, *Xba*I) that are known to be diagnostic between *S. alpinus* and *S. namaycush* (Grewe et al. 1990), as well as *Dra*I. Digested DNA fragments were visualized by Southern blotting followed by probing with radiolabeled *S. alpinus* mtDNA and autoradiography (Sambrook et al. 1989). DNA fragment sizes were determined by comparison against known size standards.

Morphometric analyses of fish were carried out using a four-box truss network (Bookstein et al. 1985). Photographic slides taken during collections were projected against a translucent screen, and distances among ten anatomical landmarks (Fig. 1A) were measured with calipers. The distance measurements were converted to proportions of the projected fork length and arcsine-transformed prior to statistical analysis. The resulting values were used to construct an Euclidean distance matrix which was subjected to multidimensional scaling (MDS), using the SYSTAT statistical computer package (Wilkinson 1990).

Results

Individual fish were tentatively identified as *S. alpinus*, *S. namaycush*, or possible hybrids based on physical appearance. Eleven of the twelve lakes contained both *S. alpinus* and *S. namaycush*, while the remaining lake (Avamuktulik) contained only *S. alpinus* (Table 1). Hybrids were found in four of the eleven lakes which contained both parent species. In total, 216 *S. alpinus*, 204 *S. namaycush*, and 5 hybrids were collected from Melville Peninsula lakes in 1989 and 1990. Three additional hybrids were obtained from one of the lakes (Lailor) in 1987 by Peter Grewe (University of Windsor, Windsor, Ont.). Most of these lakes had landlocked populations, as fish migrations were prevented by waterfalls near the lake outflows, although anadromous charr were collected in two lakes (Hall and Lailor). All populations were isolated from each other, as the waterfalls also prevented fish movements between lakes with a common river drainage. Additional collections from six lakes in the vicinities of Pelly Bay, Spence Bay, and Cambridge Bay (Northwest Territories) where both species co-occurred produced four more hybrids (Table 1).

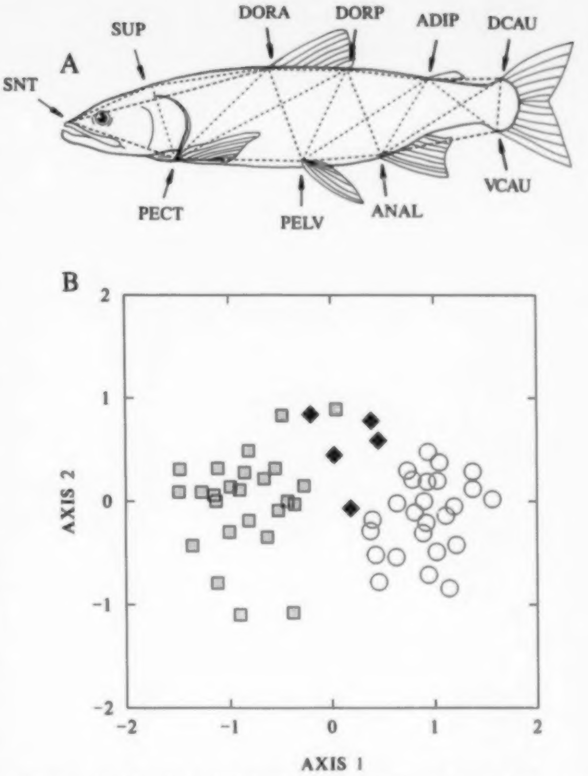

FIG. 1. (A) Morphological landmarks used to construct box truss network for comparing *S. alpinus*, *S. namaycush*, and their hybrids. Tip of snout = SNT; posterior edge of supraoccipital bone at midline = SUP; pectoral, pelvic and anal fin origins = PECT, PELV, ANAL; anterior and posterior margins of dorsal fin = DORA, DORP; anterior margin of adipose fin = ADIP; dorsal and ventral origins of caudal fin rays = DCAU, VCAU. (B) Two-dimensional plot of multidimensional scaling (MDS) of measurements resulting from box truss network analysis. Lake trout (*S. namaycush*) = ■ ; Arctic char (*S. alpinus*) = ○; hybrids = ◆.

Screening of the 1989 and 1990 Melville Peninsula populations at the 14 isozyme loci confirmed the field identifications of Arctic char, lake trout, and possible hybrids. No allozyme variation was observed for *S. alpinus*, while *S. namaycush* populations showed low levels of polymorphism for *FH** and *PGM-1**. Fixed differences were observed between the two species at seven loci (*AK-2**, *FDP**, *FH**, *LDH-1**, *MDH-3,4**, *PGM-1**, and *SOD**), with no evidence of shared alleles (Table 2). The 12 fish tentatively identified as hybrids were each heterozygous at all of these loci, which confirmed their status as F_1 hybrids.

TABLE 1. List of lakes sampled in the Canadian arctic for Arctic charr (*S. alpinus*) and lake trout (*S. namaycush*), showing lake locations and numbers of *S. alpinus*, *S. namaycush*, and interspecific hybrids collected.

Site	Latitude	Longitude	*S. alpinus*	*S. namaycush*	Hybrids
		1989 collections, Melville Peninsula			
Avamuktulik	69°15′N	82°30′W	51		
Hall Lake	68°40′N	82°30′W	29	23	
Lailor Lake	69°17′N	82°50′W	27	27	1
Sarcpa Lake	68°32′N	83°15′W	24	29	1
		1990 collections, Melville Peninsula			
Empty Lake[a]	69°15′N	84°48′W	1	4	
Grinnell Lake	69°34′N	83°55′W	10	15	
Hall Lake	68°35′N	82°40′W	22	5	
Hills Lake[a]	69°18′N	84°10′W	12	10	
Ice Lake[a]	69°16′N	84°40′W	11	16	2
Prince Lake[a]	69°34′N	84°11′W	15	14	
Tremblay Lake	69°18′N	83°20′W	5	25	
Van Raay Lake[a]	69°16′N	83°38′W	13	14	1
		Other 1990 collections			
Pelly Bay 1	68°33′N	89°47′W	8	11	2
Spence Bay 1	69°34′N	93°30′W	35	8	1
Cambridge Bay 1	69°08′N	104°49′W	17	1	
Cambridge Bay 2	69°09′N	104°43′W	25	5	
Cambridge Bay 3	69°07′N	105°07′W	25	16	1
		Other hybrid sites			
Steffanson Island[b]	75°15′N	105°12′W	3	13	1
Melville Pen.[c]	68°56′N	83°20′W	31	2	2
Melville Pen.[c]	68°49′N	84°35′W	25	5	1
Mineau River, M.P.[c]	68°27′N	85°07′W	8	17	1
Souter Lake, M.P.[c]	68°08′N	84°31′W	6	13	1
Tasialuk Lake[d]	56°44′N	62°42′W	58	33	2

[a] These site names are unofficial, and were given only to simplify sample identification.
[b] Stewart and Bernier (1982).
[c] Stewart and Bernier (1984).
[d] Hammar et al. (1989).

TABLE 2. Enzyme systems examined by cellulose acetate electrophoresis, showing IUNBC numbers, loci, source tissue, buffers used, alleles detected in *S. alpinus* and *S. namaycush*, and hybrid genotypes at diagnostic loci.

Enzyme	E.C. No.	Locus	Tissue	Buffer[a]	*alp.*	*nam.*	Hybrids
Adenylate kinase	2.7.1.20	_AK-2*_	Muscle	CAEA	0[b]	100[b]	0/100
Fructose-1,6-diphosphate	3.1.3.11	_FDP*_	Kidney	TG	100	108	100/108
Fumarate hydratase	4.2.1.2	_FH*_	Liver	TG	100	140	100/140
Lactate dehydrogenase	1.1.1.27	_LDH-1*_	Muscle	TG	100	75	75/100
		LDH-2*	Muscle	TG	100	100	
Malate dehydrogenase	1.1.1.37	_MDH-1*_	Liver	CAEA	100	100	
		MDH-2*	Liver	CAEA	100	100	
		MDH-3,4*	Muscle	TG	100	60	60/100
Malic enzyme (NADP^+)	1.1.1.40	_ME*_	Muscle	CAEA	100	100	
Phosphoglucomutase	5.4.2.2	_PGM-1*_	Muscle	TG	100	20,80	20/100
		PGM-2*	Muscle	TG	100	100	
Glucose-6-phosphate isomerase	5.3.1.9	_GPI-1*_	Muscle	TG	100	100	
		GPI-2*	Muscle	TG	100	100	
Superoxidase dismutase	1.15.1.1	_SOD*_	Liver	TG	100	130	100/130

[a] TG = Tris glycine, pH 8.5; CAEA = Amine citrate buffer, pH 7.2; buffers described in Hebert and Beaton (1989).

[b] The allele present in *S. namaycush* was assigned a value of 100, as the *S. alpinus* allele remained at the origin.

TABLE 3. Mitochondrial DNA haplotypes produced by restriction digests of *S. alpinus*, *S. namaycush*, and *S. namaycush* × *alpinus* hybrid mtDNA. Haplotype A = *S. alpinus*; haplotype B = *S. namaycush*. One hybrid possessed haplotype A (*S. alpinus*) and 11 hybrids had haplotype B (*S. namaycush*).

Enzyme	Fragment sizes									
*Ava*I										
A	6400	2680	2280	1680	1360	1100	600	570		
B	5490	2680	1840	1680	1520	1360	1100	600	570	370^{a}
*Bam*HI										
A	16800									
B	9480	5000	2320							
*Dra*I										
A	7050	3300	3070	2280						
B	7050	5350	3300							
*Hind*III										
A	9100	3440	2240	1760	260^{a}					
B	9100	2240	1960	1760	1480	260^{a}				
*Pst*I										
A	16500	300^{a}								
B	12280	4220	300^{a}							
*Xba*I										
A	13600	3200								
B	7900	5460	3200	240^{a}						

[a]Fragments visualized by endlabeling with ^{32}P.

No mtDNA variation was detected within either *S. alpinus* or *S. namaycush* for the six restriction enzymes, although each enzyme produced different haplotypes between the two species (Table 3). *Salvelinus namaycush* mtDNA fragment patterns generated from digestion with *Ava*I and *Bam*HI matched those of lake trout haplotype C from Grewe and Hebert (1988). Of the 12 hybrids, 11 showed RFLP patterns identical to those observed in lake trout, while the haplotype of the remaining hybrid matched that which occurred in the Arctic char.

Multidimensional scaling (MDS) of the truss network measurements for 25 lake trout and 25 Arctic char selected at random from lakes with hybrids separated *S. alpinus* and *S. namaycush* into two distinct clusters, with the five Melville Peninsula hybrids from 1989/1990 being intermediate between the clusters (Fig. 1B). The three hybrids collected in 1987 were not included, as morphometric data were unavailable. The physical appearance of the hybrids varied considerably, although the 1989/1990 hybrids all had head lengths intermediate between lake trout and Arctic char. Three hybrids had a body coloration similar to that of lake trout (dark with pale spots), but the remaining two had a more silvery appearance. All five 1989/1990 hybrids (three males and two females) had well-developed gonads and appeared ready to spawn. The hybrids compared favorably with co-occurring *S. alpinus* and *S. namaycush* for body size (Fig. 2). The two Ice Lake hybrids were among the largest fish collected at that site, as were the two from Sarcpa Lake. Examination of otoliths from 20 of the Ice Lake fish showed a range in age of 6–34 yr, with the two hybrids having ages of 23 and 25 yr.

Allozyme and mtDNA analyses of biopsies taken from 393 fish from Sarcpa Lake and Lailor Lake in 1991 provided conclusive evidence of introgression between *S. namaycush* and *S. alpinus*. In addition to detecting six more hybrid fish (three from each lake), allozyme screening of five of the diagnostic loci used in 1989/1990 (*AK-2**, *FH**, *LDH-1**, *MDH-3,4**, and *PGM-1**) detected appreciable levels of previously species-specific alleles in the other species for *PGM-1**. Eleven of 119 Arctic char in Sarcpa Lake and 27 of 140 in Lailor Lake had the lake trout *PGM-1*20* allele, while 9 of 93 Sarcpa Lake lake trout and 7 of 35 from Lailor Lake had the Arctic char *PGM-1*100* allele. Such extensive allele sharing was not observed at the other loci, although two Arctic char from Lailor Lake were heterozygous at *MDH-3,4* (60/100)* as well as at *PGM-1**. RFLP analyses of mtDNA from the hybrids and aberrant fish from both populations ($n = 54$) with two restriction enzymes (*Bam*HI and *Dra*I) detected 4 *S. alpinus* individuals

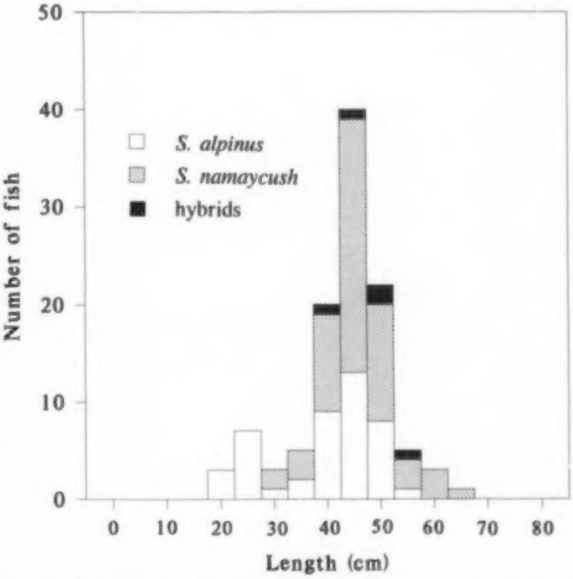

FIG. 2. Length distributions of fish collected from lakes containing hybrids in 1989 and 1990 (Ice Lake, Sarcpa Lake, and Van Raay Lake).

with *S. namaycush* haplotypes. No *S. alpinus* mtDNA patterns were detected among the *S. namaycush* screened. All six hybrids had the *S. namaycush* mtDNA haplotype.

Discussion

Although salmonid hybrids are readily produced under artificial conditions (Hubbs 1955; Buss and Wright 1956; Chevassus 1979), natural hybridization is extremely rare (Verspoor and Hammar 1991). Hybrid crosses in the genus *Salvelinus* have been carried out for more than a century (Day 1884; Stenton 1952; Alm 1955) and most of the possible interspecific crosses have been successfully attempted (Chevassus 1979). With the notable exception of splake (*S. namaycush* × *S. fontinalis*) (Stenton 1952; Ayles 1974; Berst et al. 1980), however, few studies have used lake trout in hybrid crosses.

The morphological intermediacy of the Melville Peninsula hybrids between *S. alpinus* and *S. namaycush* corresponds with the results obtained by Hammar et al. (1989), and supports the data of Stewart and Bernier (1982, 1984) of suspected hybridization between these species at other sites in the Canadian arctic. Intermediate morphology of hybrids between parent species is common among fish taxa (Hubbs 1955), and has been documented in many salmonid crosses (Alm 1955; Buss and Wright 1956), including splake (Stenton 1952; Berst et al. 1980) and sparctic char (Sutterlin et al. 1977).

Diagnosis of hybrids was simplified by the low levels of genetic variation observed among *S. alpinus* and *S. namaycush* populations with both isozymes and mtDNA. Prior work has shown that these species generally have low levels of allozyme variation in other portions of their ranges (Dehring et al. 1981; Nyman et al. 1981; Hindar 1986), although Ihssen et al. (1988) found abundant allozyme polymorphisms among 27 Great Lakes area populations of *S. namaycush*. The diversity of lake trout mtDNA haplotypes from this same region suggests that *S. namaycush* populations in the Great Lakes region originated from three separate glacial refugia (Grewe and Hebert 1988). By contrast, the low levels of allozyme polymorphism and lack of mtDNA variation in the Melville Peninsula lake trout and Arctic char suggests that each of these species originated from a single refuge.

The present study detected hybrids between *S. alpinus* and *S. namaycush* in four of the 11 Melville Peninsula lakes sampled where the species co-occurred. Three of these lakes were landlocked, while the fourth contained both resident and anadromous fish. The lack of connectivity between these lakes, as well as the four additional Melville Peninsula sites where hybrids were observed by Stewart and Bernier (1984), indicates that hybridization is occurring at a number of sites in this area. Furthermore, the occurrence of *S. namaycush* × *S. alpinus* hybrids in the western Canadian arctic (Stewart and Bernier 1982; this study) and Labrador (Hammar et al. 1989) strongly suggests that hybridization between the two species is a widespread phenomenon.

The geographically widespread occurrence of hybrids between lake trout and Arctic char might be expected, given the northern distributions of both species. Hubbs (1955) suggested that hybridization between fish species is likely to occur in recently deglaciated areas, and recent reviews of hybridization support this view (Billington and Hebert 1991; Verspoor and Hammar 1991). Most of the geographic area where lake trout and Arctic char now co-occur was deglaciated less than 9 000 yr ago (Dyke and Prest 1987), and the Melville Peninsula was covered until 6 000–5 800 yr ago (Dredge 1991). From this view, the recent formation of the lake habitats occupied by both species, as well as the species' ecological plasticity, makes hybridization extremely likely.

Hammar et al. (1989) suggested four different scenarios which could lead to hybridization between *S. alpinus* and *S. namaycush*, three of which allowed bidirectional hybridization. Their fourth hypothesis assumed *S. namaycush* to be the maternal parent, either as a result of late spawning by female *S. namaycush* or early maturation of *S. alpinus* males. This latter hypothesis seems likely, as Arctic char establish territories and spawn in pairs (Fabricius 1953), whereas lake trout spawn in groups and are nonaggressive (Martin and Olver 1980). Hammar et al. were unable to refute any of the hypotheses, however, as their morphometric and allozyme analyses did not permit determination of the directionality of hybridization. The present study, however, has made it clear that hybrids ordinarily arise as a result of matings between *S. alpinus* males and *S. namaycush* females. These matings likely resulted from the early maturation of *S. alpinus* males, as male *S. alpinus* tend to mature earlier than females (Johnson 1980).

Although bidirectional crosses between *S. alpinus* and *S. namaycush* have been carried out on hatchery fish (Chevassus 1979), the rarity of the *S. alpinus* mtDNA genome among the natural hybrids observed in this study suggests either that progeny of *S. alpinus* females by *S. namaycush* males are less fit, or that such matings rarely occur. Interestingly, Stewart and Bernier (1984) found a similar directional bias in parentage among putative hybrids, based on pyloric caecae counts of hybrids and sympatric members of both parent species. Differences in progeny survivorship in reciprocal crosses between *S. namaycush* and *S. fontinalis* have been attributed to variation in egg size, with the smaller eggs of *S. fontinalis* causing caudal deformities in hybrid embryos (Stenton 1952; Buss and Wright 1956; Berst et al. 1980). As *S. alpinus* also has an egg size slightly over half that of lake trout (Sutterlin et al. 1977; Johnson 1980; Martin and Olver 1980), similar difficulties can be expected in crosses between males of *S. namaycush* and females of *S. alpinus*.

Reciprocal crosses between lake trout and Arctic char by Sutterlin et al. (1977) showed poor hybrid survivorship, although Seguin (1957) observed excellent hatching success and good survivorship at 30 months for progeny of female lake trout and male Arctic char. Hybrids produced by crosses between brook trout (*S. fontinalis*) and Arctic char typically show hybrid vigour (Alm 1955; Sutterlin et al. 1977), as do brook trout × lake trout crosses (Berst et al. 1980). In our study, the large size of hybrids in relation to co-occurring Arctic char and lake trout (Fig. 2) suggest they are not disadvantaged for growth rate or resource acquisition. The ages of the two Ice Lake hybrids as well as the gonadal development of all 1989 and 1990 hybrids also indicate that these fish reach reproductive maturity. Experimental hybrids between lake trout and Arctic char (Chevassus 1979) also showed normal gonadal development and gametogenesis, although hybrid fertility was not examined.

Bidirectional crosses between Arctic char and brook trout produced fertile F_1's (Alm 1955; Chevassus 1979), with subsequent generations having fitnesses comparable to those observed in similar generations of splake (Ayles 1974; Berst et al. 1980). As F_2's and later generations generally showed reduced fitness in the hatchery populations (Alm 1955; Chevassus 1979), natural backcrossed and $F_{n>1}$ fish may have similar difficulties. Hammar et al. (1991), however, provided evidence of both F_2's and backcrossed fish in natural populations of brook trout and Arctic char in northern Labrador.

The combined allozyme and mtDNA analyses of fish collected in 1991 revealed that bidirectional nuclear introgression was occurring at low levels. Although Hammar et al. (1989) did not detect nuclear introgression in the Labrador population containing lake trout × Arctic char hybrids, they did observe the *PGM-1*20* allele in several other Arctic char populations in Labrador. Introgression was also detected between sympatric Arctic char and brook trout in northern Labrador (Hammar et al. 1991). The unidirectional introgression of mtDNA matches the asymmetric parentage of the hybrids collected. The presence of the *S. namaycush* mtDNA haplotype in several members of *S. alpinus* also makes the possibility of the shared PGM alleles being due to ancestral polymorphism extremely remote, and confirms that introgression has indeed occurred. The asymmetry in levels of introgression between PGM-1 and the other allozyme loci therefore suggests that F_2's and backcrossed fish face strong selective pressures, although evidently some fish are able to reproduce. Evidence from other *Salvelinus* populations (Bernatchez and Wilson, unpublished data) also indicates that transferred genes may provide some selective value.

The results of this study support those of Stewart and Bernier (1982; 1984) and Hammar et al. (1989), and suggest that natural hybridization between *S. namaycush* and *S. alpinus* occurs throughout the range of their overlapping distributions. In combination with other evidence of hybridization between native populations of Arctic char and brook trout (Hammar et al. 1991; Bernatchez and Wilson, unpublished data), the available data suggest that natural hybridization among sympatric *Salvelinus* species is more common than was previously realized, and contrasts strongly with levels of hybridization in other salmonid genera.

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Size Spectra of Benthic Communities in Laurentian Streams[1]

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The size spectra of benthic invertebrates in streams appear to differ from those reported in planktonic systems, but could reflect the narrow taxonomic scope of previous studies. When algae, protozoans, and invertebrates growing on rocks in three Laurentian streams (Québec) were grouped in logarithmically increasing size classes, patterns in the distribution of biomass were consistent over sites and dates, despite large differences in taxonomic composition. Peaks and troughs were observed in the size spectra, but the biomass of the various size classes usually lay within an order of magnitude of the mean of all classes. The slopes of the normalized size spectra were always shallower than −1 (median = −0.91) indicating a slight biomass increase in the larger size classes. Strongly peaked spectra previously observed for stream invertebrates were smoothed by the inclusion of algae and protozoans. Comparison with size spectra described for benthic and planktonic communities in lakes and oceans, showed remarkable similarities among habitats. These findings suggest that roughly similar biomass in logarithmic size classes is a general community pattern not restricted to the peculiarities of plankton communities. A size-based approach could be useful to simplify the description of complex stream communities.

Les spectres de taille des invertébrés benthiques trouvés dans des cours d'eau semblent différer de ceux signalés pour des systèmes planctoniques; mais cela pourrait refléter la portée taxonomique réduite des études antérieures. Lorsque les algues, les protozoaires et les invertébrés qui vivent sur les pierres de trois cours d'eau des Laurentides (Québec) sont regroupés en classes de taille selon une progression logarithmique, on constate que l'allure que prend la distribution de la biomasse reste constante, à différents endroits et différentes dates, malgré d'importants écarts dans la composition taxonomique. On observe des pics et des creux dans ces spectres de taille, mais la biomasse des différentes classes de taille reste habituellement à l'intérieur d'un ordre de grandeur de la moyenne de toutes les classes. Les pentes des spectres normalisés de taille sont presque toujours inférieures à −1 (médiane = −0,91); cela témoigne d'une légère augmentation de la biomasse des classes de taille les plus grandes. Les spectres à pic prononcé qui ont été associés aux invertébrés lotiques ont été atténués par l'inclusion de la biomasse des algues et des protozoaires. La comparaison avec les spectres de taille des communautés planctoniques et benthiques de lac et de mer fait ressortir des similitudes remarquables entre habitats. Ces constatations donnent à penser qu'une biomasse à peu près semblable par classe de taille logarithmique est un phénomène général des communautés, qui n'est pas restreint par les particularités des communautés planctoniques. Des méthodes fondées sur la taille pourraient permettre de simplifier la description de communautés lotiques complexes.

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Sheldon et al. (1972) proposed a model for marine planktonic communities whereby the biomass of organisms, grouped in logarithmically increasing size classes is roughly constant. Subsequently, similar size distribution spectra have been described for planktonic communities in oceans and lakes. The uniform size distribution in logarithmic size classes observed in plankton is thought to be based on a constant ratio between predator and prey size and utilization of one production level by the next (Kerr 1974; Borgmann 1987). In principle, the size approach should be unsuitable for benthic systems where algae have more varied sizes and life forms, where allochthonous food sources are significant, and where competition for space is important in structuring the communities (Strayer 1991).

In benthos, size distribution studies are centered on the invertebrates (Warwick 1984; Strayer 1986; Hanson et al. 1989; Hanson 1990; Morin and Nadon 1991; Rasmussen 1993; Poff et al. 1993) and rarely extend to protozoans and algae (Schwinghamer 1981; Cattaneo 1987). This methodological limitation is dictated by the fact that size analyses are more time consuming than in plankton because organisms have to be separated from detritus and sediments prior to measurement. Some peculiarities of benthic size distributions, like the marked differences between habitats (Strayer 1991) and the low biomasses in the smaller size classes (Morin and Nadon 1991) could simply reflect these methodological problems; some size classes could be shared by invertebrates and the large algae that can develop in attached communities.

In this study, I test the applicability of the Sheldon model of size distribution to algae and invertebrates living on stream rocks. The application of plankton models to these benthic stream communities would indicate that roughly similar biomass in logarithmic size classes is a general property of communities and not restricted to plankton where size and trophic level are more strictly coupled than elsewhere. Besides, if regularities in size spectra are found, a size-based approach could provide a

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TABLE 1. Physical and chemical characteristics at the three sampling sites. Averaged current was measured at five positions along the transect where the cobbles were collected with a Pygmy current meter held 10 cm above the stream bottom. Total phosphorus (TP) and total nitrogen (TN) analyses were performed with a Technicon instrument. TP was analyzed with the ascorbic acid modification of the molybdenum blue technique (Strickland and Parson 1972) preceded by digestion under pressure with potassium persulfate (Menzel and Corwin 1965). TN was determined by a wet-oxidation method involving peroxydisulfate digestion (Raimbault and Slawik 1991). Colour was determined by measuring the absorbance at 440 μm of water samples filtered through Gellman glass fiber filters; absorbances were transformed to standard Hazen colour units following Bowling et al. (1986).

	Cromwell		Achigan		Echo	
	May	Aug.	May	Aug.	May	Aug.
Temperature (°C)	15	21	15	19	17	24
Current ($cm \cdot s^{-1}$)	21	18	31	22	20	19
pH	6.85	6.70	7.37	7.62	8.75	8.20
Colour (Pt $mg \cdot L^{-1}$)	43	52	44	81	14	14
TP ($\mu g \cdot L^{-1}$)	20	8	21	18	17	13
TN ($\mu g \cdot L^{-1}$)	219	309	390	389	253	287

useful tool to describe stream communities and to model their energy and contaminant transfer as has been suggested for the plankton (Griesbach et al. 1982; Platt 1985; Vézina 1986).

For this test, I describe the size distribution of taxonomically different stream communities sampled at different sites and dates in the Laurentian region of Québec. These distributions are compared with those described elsewhere to assess the generality of size distribution among habitats (sea, lake, stream) and among community types (plankton and benthos).

Methods

Three second-order streams, located 100 km north of Montreal, near the Station de Biologie des Laurentides (46°N, 74°W), were sampled in May and again in August 1990: Cromwell and Echo are outlets of L. Cromwell and Echo, respectively, whereas Achigan is an inlet of Lac de l'Achigan. The sampling sites were in unshaded riffles, similar in their temperatures, currents, and low nutrient levels (Table 1). Cromwell, which is slightly acid, and Achigan, which is neutral, were both more coloured than the basic Echo (Table 1).

At each sampling, five rocks (surface area 100–300 cm^2) were collected at random from the stream bottom. Each rock was gently lifted in a dip net (100 μm mesh) placed so as to trap organisms that could be dislodged by the sampling. The rock was transferred to a plastic bowl filled with filtered water and scraped with a nylon nail brush. A 10-mL aliquot of the suspension so obtained was fixed with 4% formalin for microscopic observation of algae and protozoans. The rest of the suspension was concentrated through a 100 μm sieve, fixed with 4% formalin, and subsequently used to study the invertebrates.

Algae were counted in random fields of a nannoplankton cell at 400× and 250×. Macroalgae and ciliates were counted in a Sedgwick–Rafter cell at 100× and 25×. The length and width of 20–100 individuals of the main taxa were measured with an image analysis system connected to the microscope. This allowed calculation of individual volume by approximation to solids of similar shape with known volume and to calculate the proportion of each taxon in various sizes classes. The whole volume of colonies and filaments rather than the volume of single cells was taken as "individual" size (Cattaneo 1987). Fragments of filaments, presumably broken by brushing, were counted as fractions of an "individual" of average size.

Invertebrates were sorted under a dissecting scope at 25×. Abundant invertebrates (>100 per sample) were counted in subsamples obtained with a Folsom plankton splitter, while rarer invertebrates were counted in the entire sample. Body lengths were measured to the nearest 0.01 mm with an image analysis system connected to a dissecting scope. Individual dry mass was calculated from empirical length–mass relationships (Smock 1980; Meyer 1989). Dry mass was converted to wet mass by multiplying by 4, and to volume by assuming the specific gravity of aquatic organisms = 1.

Densities and biomasses were calculated by dividing the number of organisms or their total wet mass by the total surface area of the rock. Rock area was estimated from diameters measured along the three main axes of each rock (Graham et al. 1988).

Algae and invertebrates were grouped into logarithmic size classes corresponding to a doubling of individual "equivalent spherical diameter" (ESD) or to an approximately 8-fold increase in their wet mass. Normalized size spectra were calculated by dividing the geometric mean biomass in each size class by the width of the size class interval, and plotting these values against the upper limit of their respective size intervals (Sprules and Munawar 1986).

Results and Discussion

Size Spectra

When the algae and invertebrates were grouped into logarithmically increasing size classes, the shape of the resulting size distributions appeared similar across communities at different sites and at different dates (Fig. 1). Biomass was not evenly distributed among the size classes. Instead some classes were consistently lower (ESD 2–4 μm and 250–500 μm) or higher (ESD 16–32 μm, 1–2 mm, and 2–4 mm) than the average values. Despite these peaks and troughs, the biomass of the various size classes usually lay within an order of magnitude of the mean. It is difficult to establish if size spectra are regular because peaks and troughs are always present. Gaedke (1992) has proposed to consider smooth those spectra where "the variability of the biomass of the various size classes is small compared to the size range under investigation (1–1.5 vs. 10 orders of magnitude)." According to this definition the spectra observed

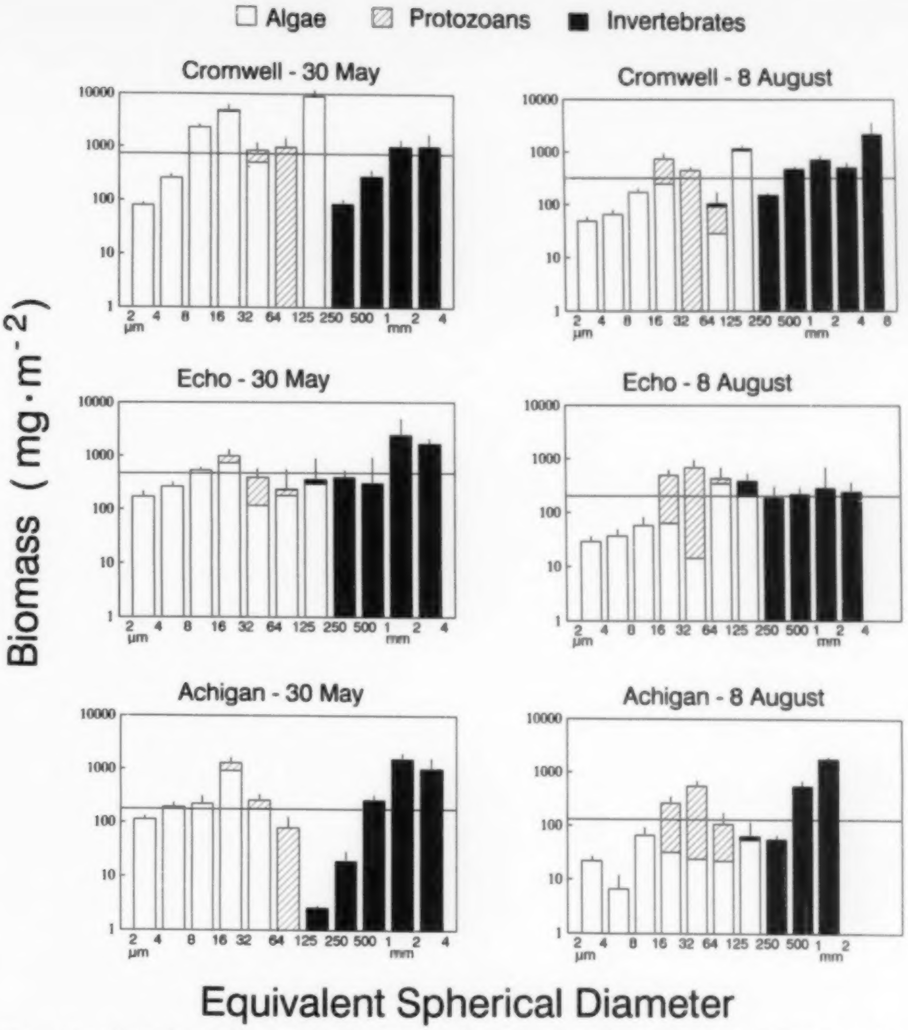

FIG. 1. Size distribution of algae (open bars), protozoans (striped bars), and invertebrates (shaded bars) growing on rocks in three Laurentian streams at two dates. Vertical bars indicate standard error where $n = 5$. The geometric means of the biomasses observed in the different size classes are represented by the horizontal lines.

at Cromwell and Echo are regular, while Achigan's are less so.

If the shape of the size distribution was remarkably similar among sites and dates, the assemblages were very different both in total biomass and in taxonomic composition. The maximum integrated biomass, observed at Cromwell in May, was about 7 times higher than the minimum value found at Achigan in August. Taxonomically (Table 2), the macroalgae (125–250 µm ESD) were represented by the green alga *Draparnaldia* and the red alga *Batrachospermum* at Cromwell in May, but by the blue-green *Stigonema* at the same site in August. At Echo, the same size class was occupied by large forms of the green *Oedogonium*. The invertebrate composition was also very variable: caddisflies and mayflies dominated the largest size classes at Cromwell, but were replaced by blackflies and planarians at Echo, whereas at Achigan, limpets were always dominant. Between 16 and 250 µm ESD, size classes were composed by both algae and invertebrates. Ciliates were important in all assemblages, sometimes filling the troughs between 16 and 125 µm ESD. The high abundance of ciliates in streams has been observed in previous studies (Bott and Kaplan 1989; McCormick 1991). Macroalgae, except at Achigan where they were rarer, occupied the size classes where previous authors (Strayer 1986; Morin and Nadon 1991; Rasmussen 1993) observed low biomasses of the smallest invertebrates.

Normalized Size Spectra

Normalized size spectra, obtained by dividing the biomass (wet mass) of each size class by the size interval and plotting it against the upper limit of the size class (Fig. 2), allow easier comparisons of distributions with each other and with those described in other studies.

There was a consistent trend in the distribution of normalized

TABLE 2. Dominant organisms in the various size classes at different sites. (M) dominant in May; (A) dominant in August; (MA) dominant on both dates. Size are expressed as equivalent sperical diameters (ESD).

ESD (μm) (except as noted)	Algae	Invertebrates
	Cromwell	
2–4	*Synecococcus* (MA)	—
4–8	*Synedra* (M), *Achnanthes*(A)	—
8–16	*Tabellaria* (M), *Gomphonema* (MA)	—
16–32	*Tabellaria* (M), *Protoderma* (A)	Ciliata (MA)
32–64	*Melosira* (M), *Bulbochaete* (A)	Ciliata (MA)
64–125	*Bulbochaete* (A)	Ciliata (MA), Chironomidae (MA)
125–250	*Draparnaldia* (M), *Stigonema* (A)	Chironomidae (MA), Oligochaeta (MA)
250–500	—	Chironomidae (MA), Trichoptera (MA)
500–1000	—	Chironomidae (MA), Trichoptera (MA)
1–2 mm	—	Trichoptera (MA), Ephemeroptera (MA)
> 2 mm	—	Trichoptera (MA)
	Echo	
2–4	*Synecococcus* (MA)	—
4–8	*Achnanthes* (MA)	—
8–16	*Tabellaria* (M), Chlorococcales (A)	—
16–32	*Synedra* (MA)	Ciliata (MA)
32–64	*Synedra* (M), *Fragilaria* (A)	Ciliata (MA)
64–125	*Oedogonium* (MA)	Ciliata (MA), Oligochaeta (MA), Chironomidae (MA)
125–250	*Oedogonium* (MA)	Chironomidae (MA), Hydracarina (MA)
250–500	—	*Hydra* (M), Oligochaeta (M), Chironomidae (MA)
500–1000	—	Simuliidae (M), Chironomidae (MA)
1–2 mm	—	Simuliidae (M), Trichoptera (A)
2–4 mm	—	Trichoptera (MA)
	Achigan	
2–4	*Synecococcus* (MA)	—
4–8	*Achnanthes* (MA)	—
8–16	*Oscillatoria* (M), *Gomphonema* (A)	—
16–32	*Oscillatoria* (M), *Cocconeis* (A)	Ciliata (MA)
32–64	*Oscillatoria* (M), *Audouinella* (A)	Ciliata (MA)
64–125	*Melosira* (A)	Ciliata (MA), Chironomidae (MA)
125–250	*Stigonema* (A)	Chironomidae (MA)
250–500	—	Chironomidae (MA), Ephemeroptera (M)

TABLE 2. (*Concluded*)

ESD (µm) (except as noted)	Algae	Invertebrates
500–1000	—	Ancylidae (MA), Trichoptera (MA)
1–2 mm	—	Ancylidae (MA), Trichoptera (MA)
2–4 mm	—	Trichoptera (M)

TABLE 3. Sizes (equivalent spherical diameters) at which peaks and troughs in the size spectra were observed in different studies and habitats.

Reference	Peaks (ESD, µm)	Troughs (ESD, µm)
Plankton — lakes		
Sprules et al. 1983	8, 500	64–125
Peters 1983b	0.45–10, 75–102	35–55, 55–75
Ahrens and Peters 1991	30, 800	200
Benthos — sea		
Schwinghamer 1981	1, 125, 16000	8, 500
Benthos — lakes		
Strayer 1986	500–1000	32
Cattaneo 1987	4–8, 1000–2000	64–125
Hanson 1990	1000–2000	16000
Benthos — streams		
Morin and Nadon 1991	8000	32
Poff et al. 1993	250, 16000	1200
This study	32–64, 2000–4000	2–4

densities over logarithmic size classes. All the regressions of log density on log size were highly significant ($p < 0.001$) and the residual variations were small ($R^2 = 0.92–0.99$). The slopes varied from −0.81 to −0.97, with a mean of −0.91 (95% CI of the mean = −0.85 to −0.97). Because the slopes were significantly shallower than −1, these distributions show a biomass increase in the larger size classes.

Some effect of sampling date could be detected. At all three sites, slopes were less negative in August than in May. Intercepts were higher in May at Cromwell and Echo, but remained the same at Achigan.

Comparisons with Other Studies

Morin and Nadon (1991) studied the invertebrate size distribution in 12 streams near Ottawa and also observed a remarkable similarity among stations despite large differences in trophy. In that study, the biomass of the small invertebrates (< 1 µg dry mass) was extremely low, resulting in bell-shaped normalized spectra. Slopes close to −1 were maintained from 100 mg to about 1 µg dry mass, but became sharply positive at smaller size classes (Fig. 3). Morin and Nadon hypothesized that this shape was not related to the peculiarities of stream environment but to the exclusions of non-metazoan organisms. My results verify their hypothesis: the smallest metazoans fall in size classes shared by macroalgae and protozoans, so that the slope becomes linear when the entire assemblage is considered (Fig. 3).

To my knowledge, only two other studies of benthic community size distribution have considered algae and protozoans as well as metazoans: a study of marine littoral communities in Nova Scotia (Schwinghamer 1981) and a study of the epiphyton on artificial macrophytes in Lake Memphremagog (Cattaneo 1987). When these distributions are compared with those observed in the Laurentian streams (Fig. 4), there is a good agreement among the three habitats. The slopes for the marine and lake communities are less negative, so the higher biomasses of the large metazoans are even more accentuated than in the streams.

These benthic size spectra also resemble those observed in planktonic communities, where spectra are always more or less jagged and slopes often shallower than −1 (Sprules and Munawar 1986; Ahrens and Peters 1991). In general, the positions of peaks and troughs are consistent within each study, but they differ among studies (Table 3). These differences could partly reflect the characteristics of the different environments. For example, differences between the position of metazoan peaks in marine and lake benthic communities could depend on the different evolutionary history of the invertebrates of these two environments (Strayer 1991). The contrast between the dominance by meiofauna and clams in the sandy bottom of a Virginian stream (Poff et al. 1993) and that by insects in several cobbled riffles of Quebec (Morin and Nadon 1991; this study) could reflect substratum and climate effects. Some differences could also be methodological, since net mesh sizes, calculation methods for organism size, etc. vary among studies.

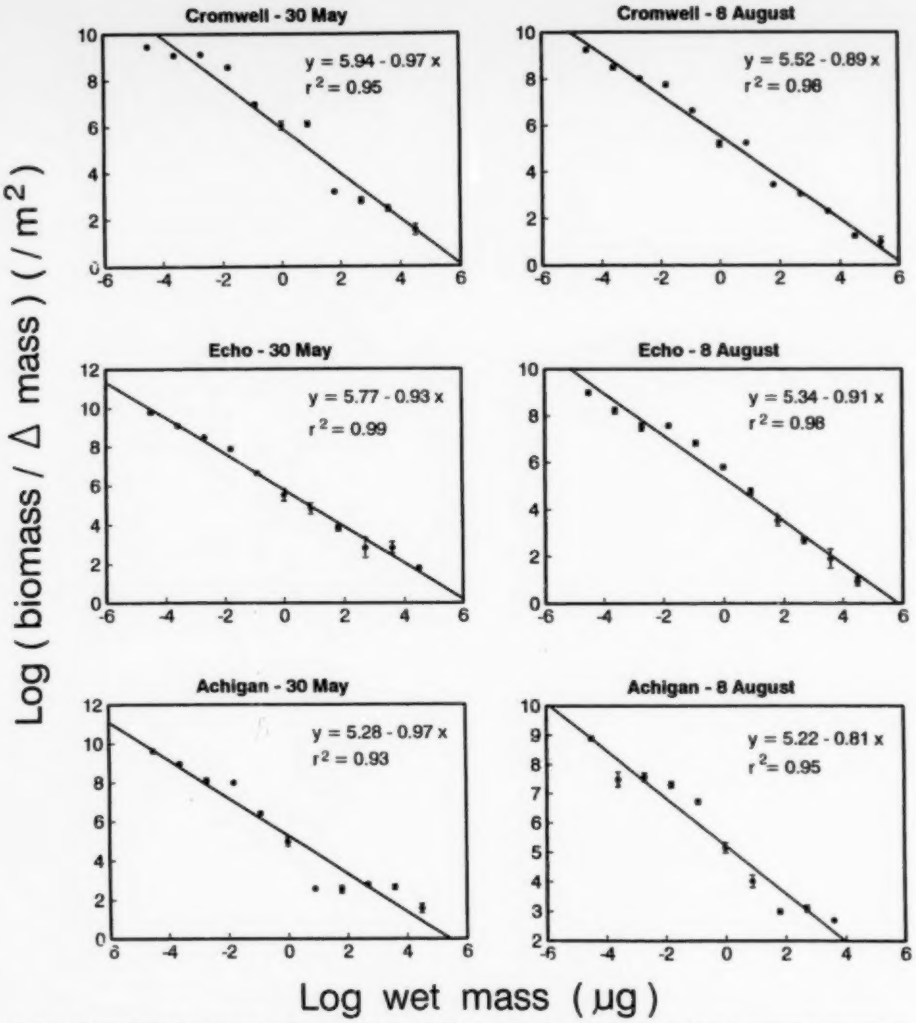

FIG. 2. Normalized biomass size spectra of the epilithic communities collected in three Laurentian streams at two dates. Each point represents the geometric mean calculated over five replicate rocks; vertical bars represent the standard error. All the regressions are highly significant ($p < 0.001$).

Conclusions

This study indicates that in benthic stream communities comprising algae, protozoans and invertebrates, biomass is roughly similar in all logarithmic size classes. Previous anomalies to this pattern were probably caused by the exclusion of algae and protozoans that share several size classes with the invertebrates rather than by the peculiarities of benthic communities. This finding suggests that, since uniform size spectra are not restricted to plankton, they do not solely depend on the regular predator-prey size ratio typical of suspended communities. These patterns may be more general since they apply to stream benthic communities that resemble terrestrial ones in the relatively large size of primary producers, the importance of detritus, the competition for space, and the availability of refuges from predators.

Because of the complexity of stream communities, taxonomic or trophic classifications are time-consuming and require a high level of taxonomic expertise. By combining size-based community descriptions with appropriate allometric relationships (Morin and Bourassa 1992), estimates of community processes could be obtained. In plankton studies, such allometric models of community structure and function have proven successful in predicting production, energetics, and contaminant flow (Griesbach et al. 1982; Platt 1985; Vezina 1986; Minns et al. 1987; Sprules et al. 1991). The strong regularities in size distribution among different sites and dates suggest that a size-based approach could similarly simplify the description of stream communities.

The similarities among size spectra observed over a wide range of habitats (sea, lakes, streams) and community types (plankton, benthos) are intriguing. If the robustness of this approach confirms the fundamental role of allometry in all aquatic communities (Peters 1983a), it puts its sensitivity to detect changes

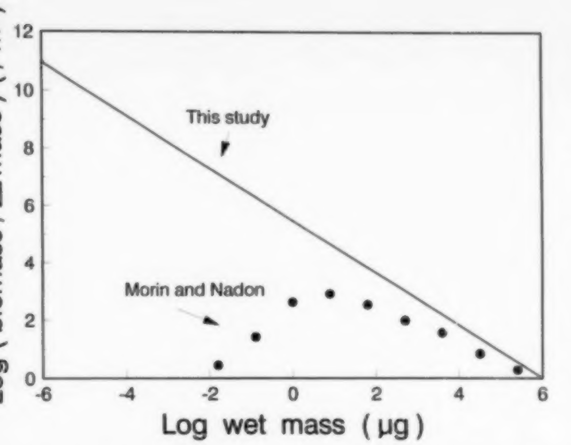

FIG. 3. Comparison between the average normalized size spectra observed in this study in 3 Laurentian streams (line), and that observed for invertebrates of 12 Outaouais streams by Morin and Nadon (1991). For the comparison, data of Morin and Nadon, originally expressed as dry mass, have been transformed to wet mass by multiplying by a factor of 4 and grouped in larger size classes.

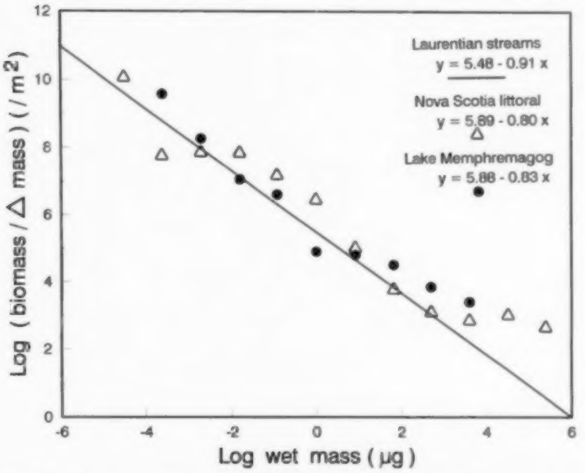

FIG. 4. Normalized biomass spectra observed in benthic community studies where algae, protozoans and metazoans were considered. Data from Schwinghamer (1981, Nova Scotia marine littoral communities) and Cattaneo (1987, Lake Memphremagog epiphyton). The line represents the average size spectrum observed in this study of Laurentian streams.

in trophy and contamination in doubt. Changes in slope and peaks have been observed between communities, but much more research is needed to test their relations with trophy, contamination, and substratum size.

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Copper and Cadmium Binding to Fish Gills: Modification by Dissolved Organic Carbon and Synthetic Ligands

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Adult fathead minnows (*Pimephales promelas*) were exposed to 17 $\mu g\ Cu \cdot L^{-1}$ or 6 $\mu g\ Cd \cdot L^{-1}$ for 2 to 3 h in synthetic softwater solutions at pH 6.2 containing either naturally-occurring, freeze-dried dissolved organic carbon (DOC) or synthetic ligands such as EDTA. After exposures, gills were assayed for bound Cu or Cd. As a first approximation, lake of origin or molecular size fraction of DOC did not influence Cu binding to gills, while DOC concentration did. DOC concentrations $\geq$4.8 $mg \cdot L^{-1}$ prevented Cu from accumulating on fathead gills. At the relatively low concentrations used, neither Cu nor Cd interfered with binding of the other metal on gills, suggesting different gill binding sites. Cadmium accumulation on gills was more sensitive to increased concentrations of Ca and H^+ than was Cu. Surprisingly, Cd bound to gills to the same or greater extent than did Cu: for synthetic ligands, Cd binds less well than Cu. This result corroborates previously published observations that Cd, unlike Cu, is taken up at gills through high affinity Ca channels. Accumulation of Cd on fish gills was never associated with ^{14}C-labelled EDTA or ^{14}C-citrate, indicating that free metal interacts with the gill while metal–ligand complexes usually do not.

Des adultes du Tête-de-boule (*Pimephales promelas*) ont été exposés à Cu en concentration de 17 $\mu g \cdot L^{-1}$ ou du Cd en concentration de 6 $\mu g \cdot L^{-1}$ pendant 2 ou 3 h dans des solutions d'eau douce synthétique à pH 6,2 qui contenaient du carbone organique dissous (COD) lyophilisé et qu'on trouve naturellement, ou des ligands synthétiques comme l'EDTA. La période d'exposition terminée, le Cu ou le Cd fixé sur les branchies, était titré. En première approximation, le lac d'origine ou la taille moléculaire du COD n'agissait pas sur la fixation du Cu aux branchies, alors que la concentration du COD avait un effet. À une concentration $\geq$ 4,8 $mg \cdot L^{-1}$, le Cu ne s'accumulait pas sur les branchies des Têtes-de-boules. Aux assez faibles concentrations testées, ni le Cu ni le Cd interférait avec la fixation des autres métaux sur les branchies; cela donne à penser qu'il existe différents sites de fixation sur les branchies. Plus que le Cu, l'accumulation de cadmium sur les branchies était davantage sensible aux hausses de concentration du Ca et du H^+. Fait surprenant, le Cd se fixait aux branchies autant ou même plus que le Cu : avec les ligands synthétiques, le Cd se fixe moins bien que le Cu. Ce résultat vient confirmer des observations dont il a déjà été fait mention, à l'effet que le Cd, contrairement au Cu, est absorbé au niveau des branchies par des voies à très forte affinité pour le Ca. L'accumulation de Cd sur les branchies des poissons n'a jamais été associée à l'EDTA marqué au ^{14}C ou au citrate marqué au ^{14}C; cela indique que les atomes métalliques libres passent en interaction avec les tissus des branchies alors que les complexes de métaux–ligands ne le font habituellement pas.

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Waterborne metals generally show their greatest toxicity to aquatic organisms in soft water of low alkalinity, low pH, and low dissolved organic carbon (Sprague 1987; Flemming and Trevors 1989; Spry and Wiener 1991). This relationship can be explained by the usual conceptual model of metal toxicity, which considers the free ion to be the toxic form of the metal (Morel 1983; Pagenkopf 1983). In essence, waterborne free metal ions must adsorb to gills before they can either exert their toxic effect at the gill surface directly, or pass through the gills on their way to internal sites of toxic action (Simkiss and Taylor 1989). Any process that prevents initial adsorption on the gill surface by reducing either the ambient free metal ion concentration, or the number of surface binding sites on the gill, will reduce toxicity of waterborne metals.

A number of conditions can affect the degree to which metals bind to gill surfaces. For instance, hardness cations such as Ca^{2+} and Mg^{2+} compete for metal ion binding sites, and metal complexing agents such as dissolved organic carbon (DOC) may reduce the free metal available to interact with an organism (Morel 1983; Pagenkopf 1983). Low water pH may also affect metal binding through H^+ competition for binding sites, and by affecting metal speciation (Campbell and Stokes 1985). In addition, H^+ can displace metals from DOC, increasing available free metal ions (Cabaniss and Shuman 1988a). A number of recent studies using fish have shown that toxicity or accumulation of both Cu and Cd varies according to competition and complexation (for Cu: Cusimano et al. 1986; Hutchinson and Sprague 1986, 1987; Playle et al. 1992; for Cd: Pärt and Wikmark 1984; Cusimano et al. 1986; Wicklund and Runn 1988; Pratap et al. 1989; Bentley 1991).

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In fish, waterborne Cu and Cd affect ion uptake through interactions at the gill. Copper (12–50 $\mu g \cdot L^{-1}$) affects ion balance by reducing Na^+ and Cl^- influx at gills (Laurén and McDonald 1985), mainly through effects on Na^+–K^+–ATPase (Laurén and McDonald 1987). High Cu concentrations (200 $\mu g \cdot L^{-1}$) increase gill permeability, as shown by increased ion efflux, probably because of displacement of Ca from intercellular tight junctions (Laurén and McDonald 1985). Cadmium also affects ionoregulation, but through interference with Ca uptake at the gill, largely through effects on a high affinity Ca^{2+}–ATPase (Verbost et al. 1989). At Cd concentrations <112 $\mu g \cdot L^{-1}$ in hard water, Na^+ influx is unaffected (Verbost et al. 1989). Because gills are the target organ of Cu and Cd, any study of the mitigating effects of water hardness or DOC should measure changes in the actual amount of metal accumulating on the gills.

In a previous study (Playle et al. 1992) we characterized the effects of H^+ and Ca competition, and EDTA complexation, on Cu binding to gills of fish held in soft water. Our first objective of the present paper was to expand the previous work to include Cd, and to examine the effects of naturally occurring DOC on both Cu and Cd binding to gills. Our second objective was to estimate metal–gill stability constants for Cu and Cd, by comparison to ligands of known metal binding ability. Our method of using complexing ligands to determine metal–gill stability constants is based on the premise that complexed metal does not react at fish gills, while free metal does. We verified this assumption using Cd and ^{14}C-labelled EDTA and ^{14}C-labelled citric acid. Detailed calculation of the metal–gill binding constants, their incorporation in a model, and testing of the model with fish exposed to Cu and Cd in natural soft water, are subjects covered in the companion paper (Playle et al. 1993).

Materials and Methods

Experimental Protocol

Adult fathead minnows (*Pimephales promelas* Rafinesque) were raised from eggs in hard groundwater at the University of Waterloo (Ca = 2860 μM, = 290 $mg \cdot L^{-1}$ as $CaCO_3$). At least 2 wk before each experiment fish were transferred to 20-L containers with aerated synthetic soft water, that was produced by adding NaCl and $CaCl_2 \cdot H_2O$ (to reach final Na and Ca concentrations of ~50 μM) to water from a mixed bed deionizer (Anderson Water Systems Ltd., Dundas, ON). Mean ± 1 S.E.M (*n*) water conditions during acclimation were: Na, 163 ± 10 (26) μM; Ca, 143 ± 9 (26) μM; pH, 6.23 ± 0.07 (26); temperature, 18.5 ± 0.4 (24)°C; Cu, 2.5 ± 0.3 (22) $\mu g \cdot L^{-1}$; and Cd, <0.1 $\mu g \cdot L^{-1}$ (17). Fish were fed daily with frozen brine shrimp.

For each experiment, 3–6 acclimated fish (0.5–4 g) were placed randomly in 1 or 2 L of aerated soft water in polyethylene containers, to which was added $CaCl_2 \cdot 2H_2O$ (Mallinckrodt; Paris, Kentucky), 1 N H_2SO_4, freeze dried natural dissolved organic carbon (DOC), or various ligands, depending on the experiment (see below). Background Cu was 2.1 ± 0.3 (32) $\mu g \cdot L^{-1}$; with added Cu (as $CuSO_4 \cdot 5H_2O$; AnalaR, BDH Toronto, ON) the concentration was 17.4 ± 0.3 (114) $\mu g \cdot L^{-1}$ (= 0.27 μM). Background Cd was <0.1 (33) $\mu g \cdot L^{-1}$; with added Cd (as $CdCl_2 \cdot 2\frac{1}{2}H_2O$; Baker Chemical Co., Phillipsburg, NJ) the concentration was 5.5 ± 0.1 (63) $\mu g \cdot L^{-1}$ (= 0.05 μM). Although these Cu and Cd concentrations are ~10× higher than those usually found in soft acidified surface waters (Hutchinson and Sprague 1986; Johnson 1991; Spry and Wiener 1991), they gave measurable metal accumulation on gills within 2–3 h of exposure (see Results). For one metal mixture experiment, zinc was added as $ZnSO_4 \cdot 7H_2O$ (AnalaR, BDH) to reach 19.1 ± 1.1 (8) $\mu g \cdot L^{-1}$ (=0.29 μM); background Zn was 2.8 ± 0.5 (10) $\mu g \cdot L^{-1}$. Unless modified, exposure pH was 6.24 ± 0.01 (159), Na was 66 ± 1 (156) μM, Ca was 34 ± 1 (134) μM, and temperature was 19.2 ± 0.3 (21)°C. Exposure time was 2–3 h, which has been shown to be enough time to allow metal accumulation on gills to stabilize (Playle et al. 1992; see Results). Treatment containers were randomized, and each treatment was usually run twice.

Dissolved organic carbon (DOC) was isolated from surface waters of several lakes near Peterborough, Ontario, namely: Salmon L., Sharps Bay and Williams Bay of Jack L., Anstruther L., Loucks L., and Wolf L. (all approximately N44°42′, W78°13′). DOC from the water of Luther Marsh, near Guelph, Ont. (N43°57′, W80°26′) was also isolated. Water was collected in 20-L stainless steel containers in 1988. Tangential flow ultrafiltration (Millipore, Bedford, MA) was used to isolate DOC into three fractions: 1000–30 000 Da, 30 000 Da to 0.2 μm, and >0.2 μm. The DOC fractions were then freeze dried and stored at room temperature in polyethylene containers. The DOC fractions were converted to the soluble sodium form in Milli Q (Millipore) water in the presence of AG50W-X8 cation exchange resin (Na form, BioRad, Richmond, CA). After 20 h the solutions were filtered through 0.2 μm polycarbonate filters (Nuclepore Corp., Pleasanton, CA). The concentrated filtrate was added directly to the aerated synthetic soft water used in the metal exposure experiments. Water DOC concentrations were measured, after acidification and sparging to remove inorganic carbon, using a Beckman model 915B total carbon analyzer. We used DOC concentrations of <8 $mg \cdot L^{-1}$ (= <8 $mg\ C \cdot L^{-1}$), that are representative of soft lake waters (Spry and Wiener 1991).

In experiments to determine metal–gill binding constants, we used the ligands ethylenediaminetetraacetic acid (EDTA; disodium salt; Baker analyzed reagent), nitrilotriacetic acid (NTA; disodium salt, Sigma Chem. Co., St. Louis, MO), ethylenediamine (EN; Sigma), citric acid (AnalaR, BDH), glutamic acid (Baker), oxalic acid (AnalaR, BDH), and salicylic acid (Fisher). These ligands were chosen to ensure a wide range of metal complexation strengths. Ligands were prepared as 1 mM stock solutions in deionized water. Calculations of free and ligand-bound metal concentrations were made using MINEQL$^+$ (version 2.1; Schecher 1991) on an IBM-compatible computer, using measured pH (fixed) and water chemical concentrations. Equilibrium stability constants at zero ionic strength (K_o, given as K in this paper) were converted to values appropriate to the ionic strength of our water ($I \sim 1 \times 10^{-4}$) by the computer program. Equilibrium constants were from the MINEQL$^+$ program, and are summarized in Table 1.

At the end of each experiment the fish were killed with a blow to the head, both sets of gills (= gill baskets) were excised, and the gills were rinsed for 10 s in 100 mL synthetic soft water. The gills were then weighed (mean wet weight = 0.07 g) and digested

TABLE 1. Log of equilibrium constants (1:1 binding to ligands) from the MINEQL$^+$ computer program. EN = ethylenediamine.

	EDTA	NTA	EN	Citrate	Oxalate	Glutamate	Salicylate
Cu-ligand	18.8	13.1	10.5	7.3	5.1	8.3	10.6
Cd-ligand	16.3	9.4	5.6	5.3	3.4	4.8	6.7
Ca-ligand	12.5	8.2	0.1	4.7	3.6	2.5	—
H-ligand	10.0	10.3	10.0	6.3	4.2	10.0	13.4

for 8 h at 80°C in 5× their weight of 1 N H_2SO_4. Gill digests were vortexed, centrifuged, and the supernatent diluted 20× with deionized water. For the NTA-Cd and citrate-Cd experiments, run 6 mo after all other experiments, 1 N H_2SO_4 added to gill samples was 10× the gill weight, with a later 10× dilution with deionized water. For all experiments, gill and water Cu and Cd concentrations were measured by graphite furnace atomic absorption spectroscopy (AAS; Varian AA-1275 with GTA-95 atomizer) using 10-μL injection volumes, N_2 gas, and standard operating conditions documented by Varian. Water Na and Ca concentrations were measured using a Perkin Elmer 4000 AAS, Zn concentrations using graphite furnace AAS, and pH by a Radiometer PHM82 meter with GK2401C combination electrode.

We compared Cu and Cd concentrations on gills of fish exposed to modified soft water with those of all fish exposed to metals in standard soft water conditions (grey horizontal bands in Figures) and with those of all unexposed fish (dashed horizontal band in Figures). Comparisons of gill metal concentrations were made using ANOVA followed by the Tukey-Kramer procedure. Data are plotted in the Figures as means ±95% CI.

^{14}C-Ligand Experiments

Experiments were run to determine the contribution of free versus ligand-bound metal to the metal load of fish gills. If only free metal binds to gills, then the metal alone would be detected in gill samples. If metal complexed by a radiolabelled ligand binds with gills, then both the metal and radiolabel would be detected. We used ^{14}C-EDTA (tetrapotassium salt) and 1,5-^{14}C-citrate for these experiments (Sigma; specific activity 11.8 and 60 mCi · mmol^{-1}, respectively). Cadmium was chosen as the test metal because less is needed for detectable accumulation in fathead gills, compared to Cu, using graphite furnace AAS.

The ^{14}C experiments were run in a similar manner to those outlined above. Two or 3 minnows were placed in 1 L of synthetic unaerated soft water for 2–3 h. The exposure water contained 0 or 0.05 μmol Cd (6 μg · L^{-1}), 0 or 0.1 μmol ^{14}C-EDTA, or 0 to 250 μmol mixtures of ^{14}C-citrate and "cold" citrate. We aimed for ~10^7 disintegrations per minute (DPM) of ^{14}C · L^{-1}, to yield reasonable counts in the gill samples. Gills were excised as before, weighed, and digested for 8 h at 80°C in 10× their weight of 1 N H_2SO_4. Digests were vortexed and 100 μL was diluted 10× with deionized water before Cd analysis by graphite furnace AAS. Remaining solutions (0.4–1 mL) were added to 16 mL of Cytoscint ES fluor (ICN Biomedicals Inc., Irvine, CA) in 20-mL plastic scintillation vials (Fisher). The vials were shaken, and then counted in a Beckman LS 1701 liquid scintillation counter with automatic quench correction. The ^{14}C label in water was determined by counting a 1-mL water sample in 16 mL of fluor.

To test detection of ^{14}C in the gill samples, five gill samples giving background counts were spiked with 10–200 μL of ~2 × 10^6 DPM ^{14}C-citrate · L^{-1} solution. In addition, ^{14}C-labelled anthracene (Sigma), a lipophilic polyaromatic hydrocarbon, was used to measure the uptake of a ^{14}C-compound known to cross biological membranes. Ten microlitres of 0.1 μCi ^{14}C-anthracene · μL^{-1} (=0.07 μmol) in dimethyl sulphoxide carrier (DMSO; Omnisolv, BDH) was added to 1 L of synthetic soft water, to yield ~2 × 10^6 DPM · L^{-1}. Three minnows were held in this water for 2 to 3 h. Ten microlitres of DMSO in 1 L was tested with ^{14}C-citrate (~4 × 10^6 DPM · L^{-1}) to determine if DMSO contributes to citrate entry into gills. Gill and water samples were counted as before.

Because citric acid is a substrate in the tricarboxylic acid cycle, it was possible that ^{14}C-citrate bound by fish gills could be metabolized to $^{14}CO_2$ and expired. This possibility was tested using 250-mL solutions in sealed jars with and without ^{14}C-citrate, Cd, and fish. Water in the jars was acidified after 2 h to pH 3.7–4.0 with 1 N H_2SO_4 to liberate CO_2 from the test waters. Containers, with pleated pieces of filter paper soaked in 1 mL of 10% KOH, were suspended inside the jars to trap evolved CO_2. Filter papers plus the KOH were added to 16 mL of fluor, and 200 μL of glacial acetic acid was added to each sample to reduce chemolumescence. Samples were counted after storage in the dark overnight.

Results

Time Course and Effects of H$^+$ and Ca on the Accumulation of Cd on Minnow Gills

Cadmium accumulation on fathead minnow gills was linear to about 2 h ($r = 0.92$, $P < 0.001$), after which accumulation levelled off (Fig. 1). Two- to 3-h exposures were therefore appropriate for assessing initial accumulation of Cd on minnow gills. The Cd accumulation pattern was the same whether or not 0.1 μM EDTA was present at twice the Cd concentration, however no Cd accumulated on gills in 2 h if 0.25 μM EDTA (at 5× the Cd concentration) was present (Fig. 1).

Increasing water Ca concentration from 35 to 95 μM did not significantly reduce Cd accumulation, but Ca concentrations of 1055 and 2000 μM fully prevented Cd accumulation on gills (Fig. 2). In Cd exposures run at pH 4.83 ± 0.08 (7) with no additional Ca, no Cd accumulated on gills (Fig. 2).

Effects of Natural Dissolved Organic Carbon on Metal Binding to Gills

To determine if either the source of dissolved organic carbon (DOC), or the size fraction of DOC, affected Cu accumulation on gills, we tested three size fractions of DOC from five lakes and Luther Marsh. As preliminary data using Luther Marsh DOC suggested that 5 mg DOC · L^{-1} prevented Cu

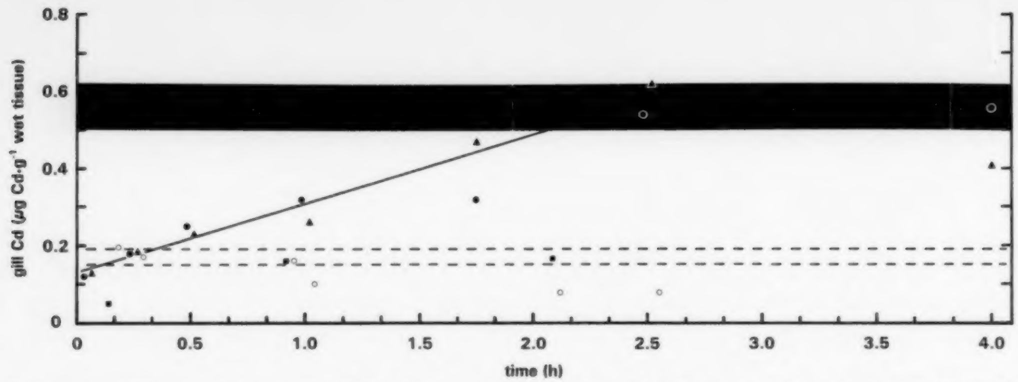

FIG. 1. Time course of Cd accumulation on gills of fathead minnows exposed to 6 μg Cd · L^{-1} (0.05 μM) in soft water (●). EDTA (0.1 μM) (▲) did not affect Cd accumulation, whereas 0.25 μM EDTA did (■). ○ = fish not exposed to Cd. ■ and ○ were only continued to 2.5 h. A straight line was fit to the ● and ▲ data to 2.5 h. Dashed horizontal lines represent the 95% CI of gill Cd of all fish not exposed to Cd (n = 38). The grey horizontal band represents the 95% confidence interval of gills of all fish exposed to 6 μg Cd · L^{-1} for 2–3 h in synthetic soft water (n = 45). Background and Cd-exposed gills had significantly different concentrations of Cd ($P < 0.001$).

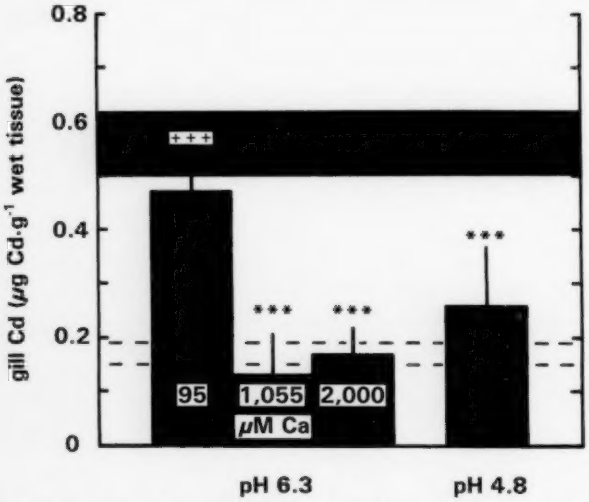

FIG. 2. Effects of Ca and H$^+$ on Cd accumulation on gills of minnows exposed to 6 μg Cd · L^{-1} for 2 to 3 h in soft water (pH 6.2). ≥1,055 μM Ca prevented Cd accumulation on gills. No Cd accumulated on gills of fish held at pH 4.8 (no added Ca). Vertical lines represent the 95% confidence interval (from left to right, n = 5, 6, 6, 5). For all figures, *, **, *** = significantly below metal-exposed gills (grey horizontal band) and +, ++, +++ = significantly above background gill metal concentrations (dashed horizontal lines) for $P < 0.05$, < 0.01, < 0.001, respectively.

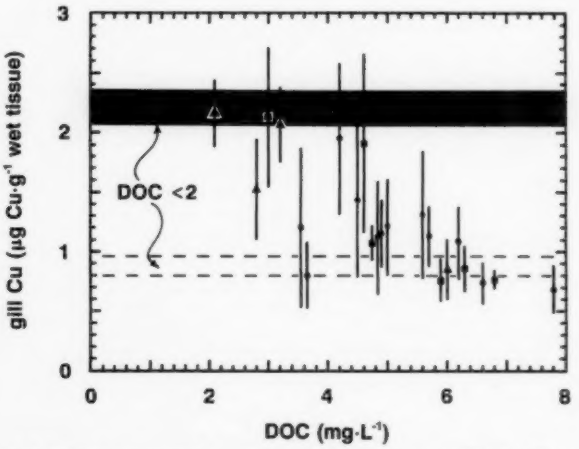

FIG. 3. Gill Cu concentrations of fathead minnows exposed for 2–3 h in soft water (pH 6.2) to 17 μg Cu ·L^{-1} and various concentrations of dissolved organic carbon (DOC). Three size fractions of freeze-dried DOC from various lakes were used: ● = 1000–30 000 Da, ■ = >30 000 Da but <0.2 μm, and ▲ = >0.2 μm. Gill Cu was reduced significantly at all DOC concentrations ≥4.8 mg · L^{-1}. The data fit a straight line: gill Cu = −0.26 · [DOC] + 2.52 ($r = -0.77$, $P < 0.001$). Vertical lines represent the 95% confidence interval (n = 5 to 6 fish gills). Dashed horizontal lines represent the 95% CI of background gill Cu (n = 67). The grey horizontal band represents the 95% CI of all Cu-exposed fish gills (n = 74) in the absence of added DOC (DOC <2 mg · L^{-1}). Background and Cu-exposed gill Cu concentrations were significantly different ($P < 0.001$).

accumulation on minnow gills (data not shown), we tried to test that amount of each DOC fraction, although not all size fractions for all lakes were examined.

Accumulation of Cu on gills of fish exposed to 17 μg Cu· L^{-1} in the presence of DOC indicated that DOC concentration was the main factor in decreasing Cu binding to gills (Fig. 3). All gill Cu concentrations were reduced to background at DOC ≥4.8 mg· L^{-1}, regardless of DOC size source or fraction, and were significantly reduced relative to gills from fish exposed to Cu in the absence of DOC ($P < 0.001$). Below 4.8 mg· L^{-1} little or no protective effect of DOC was observed, except for two values at 3.7 mg· L^{-1}. These two results were from the 1000–30 000 Da DOC fraction from Salmon L. and Williams Bay; neither was significantly different from background gill Cu, and both were significantly lower than Cu-exposed gills in the absence of added DOC ($P < 0.001$). These two values suggest that lower molecular weight DOC fractions complex Cu to a greater degree than do higher molecular weight fractions. Otherwise it appears that, as a first approximation, DOC concentration, not source or size fraction, is the dominant factor in determining metal accumulation on fish gills.

Next, we looked at the effects of DOC on Cu and Cd

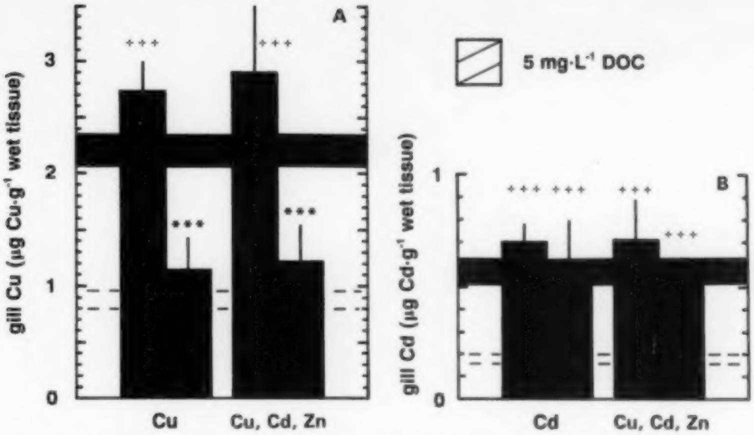

FIG. 4. (A) Gill Cu concentrations of fathead minnows held for 2–3 h in soft water (pH 6.2) with either 17 μg Cu $\cdot$ L^{-1} or 17, 6, and 19 μg Cu, Cd, and Zn $\cdot$ L^{-1}, in the presence (stripes) or absence of DOC. Five milligrams DOC per litre reduced gill Cu to background; (B) gill Cd concentrations of fathead minnows held for 2–3 h in soft water (pH 6.2) with either 6 μg Cd $\cdot$ L^{-1} or 17, 6, and 19 μg Cu, Cd, and Zn $\cdot$ L^{-1}, in the presence or absence of DOC. Five milligrams DOC per litre (stripes) did not significantly reduce Cd accumulation on the gills.

accumulation on minnow gills, either present as individual metals or as a mixture of Cu, Cd, and Zn. Once again, 5 mg DOC$\cdot$ L^{-1} (1000–30 000 Da Luther Marsh) prevented Cu accumulation on fathead gills (Fig. 4A). The simultaneous presence of 6 μg Cd$\cdot$ L^{-1} and 19 μg Zn$\cdot$ L^{-1} did not affect Cu deposition, in either the absence or presence of DOC. This result indicates either strong binding of Cu to the gills (relative to Cd and Zn), excess metal binding sites on the gills, or different binding sites for each metal. Background gill Zn concentrations were so high (20–40 μg Zn$\cdot$ g wet tissue^{-1}) that the 19 μg Zn$\cdot$ L^{-1} exposures did not significantly alter gill Zn concentrations (data not presented).

Cadmium accumulation was unaffected by simultaneous exposure to Cu and Zn (Fig. 4B), again indicating either strong Cd binding, or excess or different metal binding sites on gills. With 5 mg DOC$\cdot$ L^{-1} there was no significant reduction in the amount of Cd on the gills (Fig. 4B), in marked contrast to the results for Cu (Fig. 4A). Later experiments to determine the concentration of DOC needed to prevent Cd accumulation on gills, yielded 0.29 ± 0.15 (6) and 0.30 ± 0.08 (6) μg Cd $\cdot$ g wet tissue^{-1} (±95% CI) for 5.1 and 7.7 mg DOC $\cdot$ L^{-1} of the 1000–30 000 Da Luther Marsh fraction, respectively. Both these concentrations were significantly below values from Cd-exposed fish in the absence of DOC ($P < 0.001$, <0.01, respectively), and were not significantly above background.

Complexation of Cu and Cd Using Synthetic Ligands

We used synthetic ligands to estimate conditional metal–gill equilibrium constants (K) for Cu and Cd. The approach is based on equilibrium constants for metal–ligand complexes in soft water, such as those published in Morel (1983) and used in computer programs like MINEQL$^+$ (Schecher 1991). For example, the Cu-EDTA complex ($\log K = 18.8$) forms about 300× more strongly than does Cd-EDTA ($\log K = 16.3$), and about $0.5\times10^6\times$ more strongly than does the Cu-NTA complex ($\log K = 13.1$; $\log K$ values from MINEQL$^+$, summarized in Table 1). Copper binds to NTA about 5000× more strongly than does Cd ($\log K_{\text{Cd-NTA}} = 9.4$). Note that in all cases Cu binds to these ligands better than does Cd. By exposing fish to metals and ligands at known concentrations in water of defined composition, and measuring metal accumulation on gills, metal–gill stability constants can be estimated.

Copper

To estimate Cu-gill stability constants, we exposed fathead minnows to 17 μg Cu$\cdot$ L^{-1} (0.27 μM) in soft water in the presence of various ligands. 0.25 μM EDTA prevented Cu from accumulating on gills (Fig. 5), indicating that enough Cu was bound in the Cu-EDTA complex to render Cu unavailable to bind to the gills. MINEQL$^+$ calculations yielded 0.019 μM free Cu, with the remaining Cu in the Cu-EDTA form. Likewise, 0.25 μM NTA prevented Cu accumulation (Fig. 5). Only about 0.030 μM Cu was calculated to be in the free form in the presence of 0.25 μM NTA (MINEQL$^+$ calculation). 2.5 μM NTA also reduced gill Cu to background (0.61 ± 0.16 (4)); only about 0.0002 μM Cu was calculated to be free at this NTA concentration.

Interpretation of results using ligands that bind Cu more weakly than EDTA and NTA is complicated by the fact that Ca and H$^+$ compete with Cu for these ligands (e.g. $\log K$ values are similar, Table 1). Thus, not all the added ligand is available to bind Cu. For example, only 0.04 μM of the 0.25 μM ethylenediamine was in the CuEN form (~16%), the rest of the ethylenediamine being in the H$^-$ or H^{2-} form (MINEQL$^+$ calculation). In contrast, >96% of the 0.25 μM EDTA or NTA was available to bind Cu (above). Ethylenediamine, citric, and oxalic acid (25 μM) were needed to prevent Cu accumulation on gills (Fig. 5), for which 0.006, 0.007, and 0.044 μM, respectively, of the 0.27 μM Cu in solution, was calculated to be free. In addition to these data, we had conducted a series of experiments with glutamic and salicylic acids, in which 25 μM glutamate reduced gill Cu accumulation slightly (but not significantly; to 1.84 ± 0.54 (6)), and 250 μM salicylic acid was needed to reduce gill Cu to 1.31 ± 0.28 (5), not significantly above background.

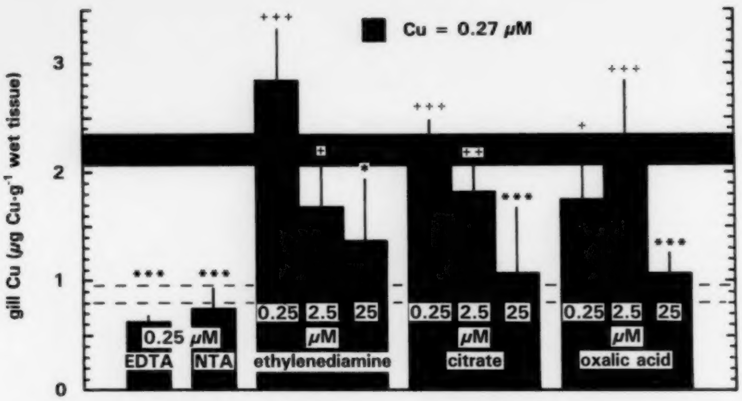

FIG. 5. Amount of Cu on the gills of fathead minnows exposed to Cu (0.27 μM = 17 μg · L^{-1}) and ligands for 2–3 h in soft water at pH 6.2. 0.25 μM EDTA and NTA prevented Cu accumulation on the gills, whereas higher concentrations of the other ligands were needed to do so. From left to right, n = 10, 4, 9, 9, 9, 6, 5, 5, 6, 5, 5. The horizontal bands designated by dashed lines or grey shading represent, respectively, the 95% CI for background gill Cu, and Cu-exposed gills in the absence of ligands.

Cadmium

The results for Cd binding to gills in the presence of synthetic ligands were surprising. Although Cd binds less well to synthetic ligands than does Cu (Table 1), Cd bound to *minnow gills* as well or better than did Cu. Between 2 and 5× more EDTA than Cd was needed to prevent Cd accumulation on gills (Fig. 6; see also the Cd time course experiment, Fig. 1). This much EDTA would leave between 0.001 and 0.005 μM of the 0.05 μM total Cd in solution as free metal (MINEQL$^+$ calculation). Extremely low concentrations of free Cd resulted in significant Cd accumulation on gills, in contrast to the 0.006 to 0.04 μM free Cu that was not enough free Cu to result in increased gill Cu concentrations (above).

Both Ca and H$^+$ complex with NTA and citric acid, reducing the amount of ligand available to bind Cd. NTA (5 μM) and 2500 μM citric acid significantly reduced Cd accumulation on minnow gills (Fig. 7). Free Cd under these conditions was calculated to be 0.03 and 0.0002 μM, respectively. The citric acid results agree with the Cd-EDTA results: virtually all Cd in solution must be complexed, otherwise measurable Cd accumulation occurred. The Cd-NTA results agree with the Cu-oxalic acid results, in that 0.03–0.04 μM of free metal was not enough to result in metal accumulation on the gills. Considering all the Cd and Cu data on a molar basis, there needs to be the same or less free Cd than Cu if no metal is to accumulate on the gills. That is, the affinity of Cd for the gills is the same or greater than that of Cu.

^{14}C-Ligand Experiments

To this point the experimental design and our interpretation of the results were based on the assumption that free metals interact with fish gills, while metal–ligand complexes do not. Copper-EDTA and Cu-NTA complexes apparently do not bind to fish gills (Fig. 5), but Cu accumulation in the presence of low concentrations of ethylenediamine, citric acid, or oxalic acid could be interpreted as Cu-ligand accumulation on or in gills. Similarly, elevated Cd accumulation on gills for the lower ligand concentrations (Fig. 6, 7) could have been due to Cd-ligand accumulation on or in gills. To ensure the correctness of our interpretations, we wanted to verify that the free metal, not the metal–ligand complex, was responsible for elevated gill metal concentrations in our experiments. Numbers of fish used in these experiments were small, due to the expense of the radiolabelled ligands.

Three fathead minnows exposed to ^{14}C-EDTA (0.1 μmol EDTA = 2.4 ×10^6 DPM) in 1 L of soft water (no Cd) had background gill Cd values of 0.11–0.13 μg Cd · g^{-1}, and 36–48 DPM per gill sample (Fig. 8), the same level of radioactivity as fish exposed to Cd without radiolabel (29–43 DPM per gill sample). Thus, ^{14}C-EDTA did not accumulate on minnow gills. In Cd plus ^{14}C-EDTA exposures, gills accumulated Cd but there was no increase in gill DPM (Fig. 8). MINEQL$^+$-calculated Cd-EDTA was 90% of total Cd. Assuming 1:1 Cd:EDTA binding, the expected counts if 90% of the Cd accumulation was due to Cd-EDTA would be 500–1 800 DPM (gill samples = 0.07 g wet weight each; allowing a generous (50%) margin for counting inefficiency and loss of sample). To ensure that these seemingly large counts would be detected, we added 10–200 μL of 0.1 μM ^{14}C-citrate (= 2.45 × 10^6 DPM · L^{-1}) to gill samples from control fish. Gill DPM versus radiolabel added was a linear relationship (r = 0.999, n = 5). A 10-μL spike (1 × 10^{-6} μmol citrate) yielded 22 DPM above background, and a 20-μL spike yielded 44 DPM above background. An additional 20–40 DPM (1.5–2× background counts) would therefore easily be detected in our system.

^{14}C-labelled anthracene, a lipophilic polyaromatic hydrocarbon, was also used to test detection of ^{14}C. About 2 × 10^6 DPM of ^{14}C-anthracene (0.07 μmol) was added to 1 L of soft water, and three fish were exposed to this solution for 2–3 h. These fish had gill counts of 370–550 DPM (Fig. 9), whereas two control fish had gill counts of 39 and 46 DPM. Two fish exposed to the dimethyl sulphoxide (DMSO) carrier plus 0.1 μmol ^{14}C-citrate (= 4.2 × 10^6 DPM · L^{-1}) had gill counts of 49 and 58 DPM (Fig. 9); anthracene's entry was therefore due to its own properties, not to DMSO. The ^{14}C-anthracene experiment also indicates that 500–1 800 DPM (^{14}C-EDTA experiment) would easily have been detected in our system.

Cadmium accumulation on gills was also never associated with ^{14}C-citrate (Fig. 10). Nine fish exposed to 0.1 μM of

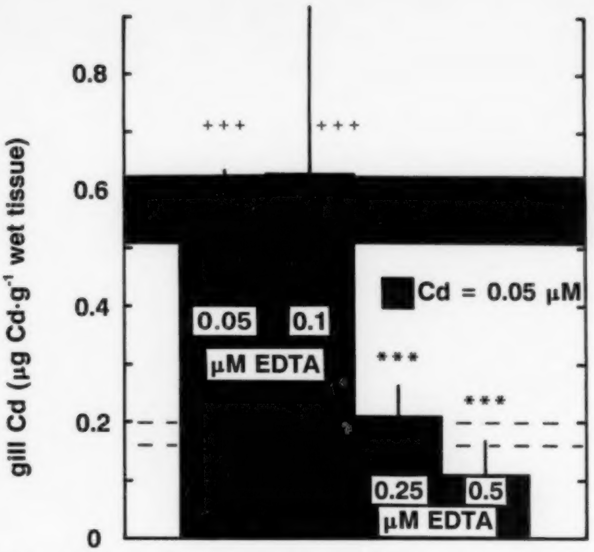

FIG. 6. Amount of Cd on gills of fathead minnows exposed to Cd (6 µg · L^{-1} = 0.05 µM) and EDTA for 2–3 h in soft water at pH 6.2. It took between 2 and 5× more EDTA than Cd to prevent Cd accumulation on the gills. From left to right, n = 13, 4, 5, 4. The horizontal bands designated by dashed lines or grey shading represent, respectively, the 95% CI for background gill Cd, and Cd-exposed gills in the absence of ligands.

^{14}C-citrate had background gill Cd concentrations and a mean gill radioactivity value of 58 DPM. Nine fish exposed to Cd alone had gill Cd concentrations of 0.31 µg Cd · g^{-1} (wet tissue) and mean gill DPM values of 44. Three fish held in 0.1 µM ^{14}C-citrate plus 0.05 µM Cd showed Cd accumulation on gills, but no ^{14}C-citrate entry. However, MINEQL^{+} calculations showed 98% free Cd under these conditions, so binding of free Cd, as opposed to Cd-citrate, would be expected. Similarly, there was no evidence of ^{14}C-citrate on gills of fish held in 5 µM citrate with 0.1 µM ^{14}C-citrate: again, plenty of free Cd would be available to bind at the gills (80% of total Cd) under these conditions.

With Cd plus 25 µM cold and labelled citrate (16.35 × 10^{6} DPM in 1 L) no counts due to ^{14}C were detected in the gill samples (Fig. 10). Here, 57% of the Cd would be bound by citrate (MINEQL^{+} calculations). Under these conditions, again with a generous provision for counting inefficiency, an additional 18, 21, and 31 DPM would have been expected in the samples if 57% of the Cd accumulation was due to Cd-citrate entry. These increases would have been detected in our system, but the gill samples were within the background range. Finally, 250 µM citrate (19.44 × 10^{6} DPM in 1 L) did not prevent Cd accumulation on the gills, in accord with results presented in Fig. 7. Here, 95% of the Cd was calculated to be in the Cd-citrate form, and 9, 6, and 6 DPM above background would have been expected if 95% of the Cd accumulation on the gills had been due to Cd-citrate. These values are at the detection limit of this method, but the gill DPM values were within background. In total, there was no evidence that ^{14}C-citrate accumulates on gills.

There was, however, the possibility that ^{14}C-citrate was taken up at the gills and metabolized to $^{14}CO_2$ in the tricarboxylic acid cycle. This scenario was tested by trapping $^{14}CO_2$ in 10% KOH in sealed jars containing 250 mL of a 0.1 µM ^{14}C-citrate solution (6.7 × 10^{6} DPM · L^{-1}). The KOH sample from three jars containing one fish each yielded 17 270 ± 1 140 DPM (mean ±1 SEM). With 6 µg Cd · L^{-1} in solution, KOH from jars containing fish yielded 13 310 ± 1 180 DPM. KOH from two sealed jars (no fish) with magnetic stir bars set to mimic agitation of water due to minnows swimming gave 18 210 and 23 820 DPM. KOH from a single, unstirred sealed jar (no fish), yielded 3 240 DPM, and KOH alone gave 80 DPM. ^{14}C caught in the KOH traps was unrelated to the presence of fish, and appeared to be due to breakdown or volatilization of the ^{14}C-citrate, encouraged by agitation of the water either by fish or by stir bars. It is therefore unlikely that metabolic breakdown of ^{14}C-citrate bound to gills was responsible for low counts of gills from the ^{14}C-citrate plus Cd experiments.

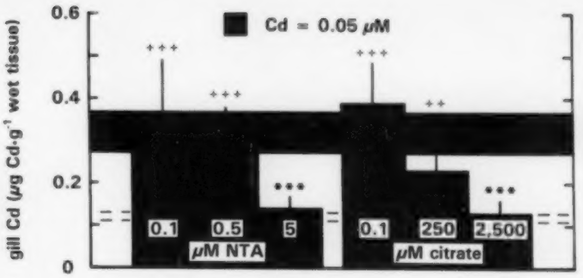

FIG. 7. Amount of Cd on gills of fathead minnows exposed to Cd (6 µg · L^{-1} = 0.05 µM) and NTA or citric acid (2–3 h in soft water, pH 6.2). NTA (5 µM) and 2500 µM citric acid were necessary to prevent Cd accumulation on gills. These experiments were done ~6 mo after those presented in previous Figures, which shifted Cd values for background (dotted horizontal lines, n = 12) and Cd-exposed gills in the absence of added ligands (grey horizontal band, n = 12). From left to right, n = 5, 5, 5, 9, 5, 5.

Discussion

The most surprising result of our work was the strong binding of Cd to fathead minnow gills relative to that of Cu. Synthetic ligand experiments (Fig. 5, 6, 7) indicated that the concentration of free Cd required for significant accumulation of Cd on gills — using graphite furnace technology — was the same or less than the concentration of free Cu required for significant Cu binding. If adsorption on the gill surface was the sole factor determining binding, and was similar to binding to other ligands, then Cd would be expected to bind about 10–100× less well to gills than would Cu (Table 1). The probable reason for the difference is uptake of Cd through high affinity Ca channels, in addition to surface binding, whereas Cu uptake is likely through general surface binding plus possible uptake through low affinity ion channels. Both Cd and Ca cations have an ionic radius of ~1.2 Å, and Verbost et al. (1989) showed that any treatment that decreased gill Ca accumulation had a similar effect on Cd accumulation, strongly suggesting that both cations enter gills by the same pathway.

Reid and McDonald (1991) also found that Cd bound to fish gills better than did Cu. Radiotracers bound to excised rainbow trout gills in the order $La^{3+} > Ca^{2+} = Cd^{2+} > Cu^{2+}$. Lanthanum was expected to bind best, due primarily to its triple charge; they explained the weaker than expected binding of Cu to gills through the coordination chemistry of Cu^{2+} (Reid and McDonald

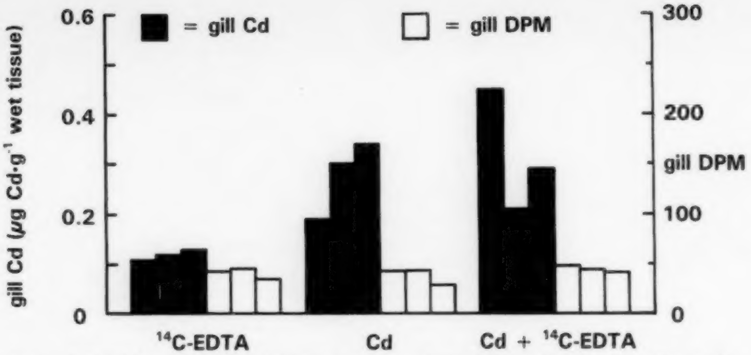

FIG. 8. Gill Cd (grey bars) and disintegrations per minute (DPM; clear bars) of gill samples from fish exposed for 2–3 h in soft water to 0.1 μM ^{14}C-EDTA, Cd (6 μg · L^{-1} = 0.05 μM), or 0.1 μM ^{14}C-EDTA plus Cd. No additional counts due to ^{14}C-EDTA were detected. Three fish per treatment, with the order of gill Cd and DPM preserved left to right in each treatment.

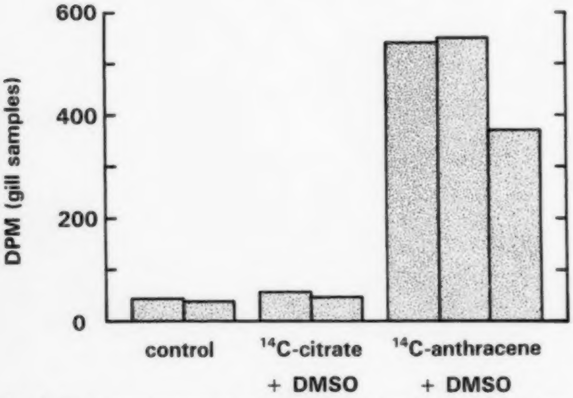

FIG. 9. Disintegrations per minute (DPM) of fathead minnow gills from two fish exposed to soft water only (control), two fish exposed to 0.1 μM ^{14}C-citrate plus dimethyl sulphoxide (DMSO), and three fish exposed to 0.07 μM ^{14}C-anthracene plus DMSO. Accumulation of ^{14}C-labelled anthracene in or on gills was easily measured by our technique.

1991). From our viewpoint, the explanation needed is why Ca and Cd bound *better* to the gills than did Cu. As presented above, active uptake of Ca and Cd at high affinity sites can explain their results as well as ours. Radiotracers such as ^{109}Cd can be used at very low concentrations of total metal (e.g., 0.003 μM Cd, about a 10× lower concentration than we used), but at such low Cd concentrations exposure times necessary for adequate gill counts need to be about one day (Wicklund Glynn et al. 1992). Therefore, graphite furnace AAS is a good method to measure metal uptake at gills, because it allows both low metal exposure concentrations and short exposure duration.

Significant morphological changes in fish gills occur at $\geq$10 μg Cd · L^{-1}, even in synthetic soft water (Karlsson-Norrgren et al. 1985). Low concentrations of Cd do not lead to general increases in gill permeability to ions (increased leakiness due to displacement of Ca from cell membranes; Verbost et al. 1989). Therefore, the ameliorative effects of Ca against low concentrations of Cd are probably due to specific Ca–Cd antagonism, not to a general reduction in gill permeability (Pratap et al. 1989; Verbost et al. 1989; Fig. 2). Reduced Ca influx (Reid and McDonald 1988) can be explained by inhibition of basolateral Ca^{2+}-ATPase. This inhibition results in increased intracellular Ca, with a resultant reduction in Ca entry at cell surfaces (Verbost et al. 1989), and may eventually lead to cell death.

Our dissolved organic carbon (DOC) results indicate, as a first approximation, that source and size fraction of DOC do not determine the protective effect of DOC against Cu binding to gills. If DOC concentration was $\geq$4.8 mg · L^{-1}, Cu was prevented from binding to minnow gills (Fig. 3). This result agrees with those of Cabaniss and Shuman (1988b), who found that source may be less important in explaining variations in Cu binding by dissolved organic matter than are chemical factors such as pH, alkalinity, and ionic strength. Cadmium binds to DOC about 10× less well than does Cu (Morel 1983; Alberts and Giesy 1983; Fig. 4). Estimates of Cu-DOC binding vary considerably, with log K values ranging from 6 to 11 for water with pH > 6 (Morel 1983). Our own estimates of Cu and Cd binding constants to DOC are made in our companion paper (Playle et al. 1993).

Our experiments were run for 2–3 h, long enough for Cd and Cu accumulation on gills to stabilize (Fig. 1; Playle et al. 1992). Measured metal accumulation is probably a combination of surface-adsorbed metal, and subsequent metal transfer across gill surfaces. This process of adsorption followed by entry is assumed to occur for metal uptake by algae (Morel et al. 1991) and is likely the process at fish gills. Verbost et al. (1989) used an EDTA rinse to remove surface-bound ^{109}Cd and ^{45}Ca, and found that both tracers were associated with the inside of the gill epithelium. No differentiation between surface-bound and interior metal was made in our study. Our premise was that any process affecting initial adsorption at the gills — mainly competition or metal complexation — would reduce initial metal adsorption and therefore accumulation and toxicity. Results of Skowroński et al. (1992) indicated that similar competition and complexation effects (by K$^+$ and Cl$^-$, respectively) are seen in either 5- or 90-min Cd exposures, even though approximately 20% more Cd accumulated in their cyanobacterium during the longer exposure.

Metal binding proteins such as metallothionein may afford some protection against Cu over time, but production of metallothionein was likely not a factor in our short experiments.

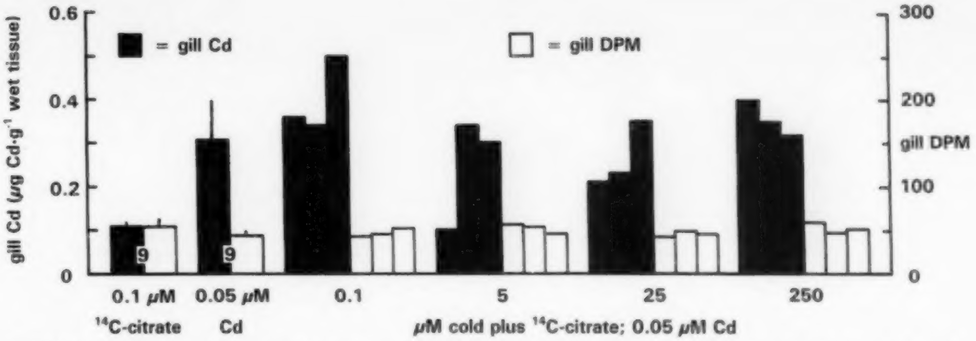

FIG. 10. Gill Cd (grey bars) and disintegrations per minute (DPM, clear bars) of gill samples from fish exposed for 2–3 h in soft water to combinations of ^{14}C-citrate, "cold" citrate, and Cd (6 $\mu g \cdot L^{-1} = 0.05$ µM). Wider bars at left represent mean values ±95% CI ($n = 9$); otherwise each bar represents one gill sample, with order preserved left to right in each treatment. There were no additional counts due to ^{14}C-citrate.

In any case, the gill is the first organ affected by waterborne Cu; induction of metallothionein-like proteins will only have protective effects once gills have regained normal regulatory function (Laurén and McDonald 1985). Longer-term studies would indicate if acclimation to Cu and Cd occurs through changes in metal–gill binding characteristics (Reid et al. 1991), changes that could lead to reduced metal concentrations on the gills (McDonald et al. 1991).

Interpretations of our results are based on the premise that some ligands can outcompete gills for metals, whereas other ligands that form weak metal complexes will not. Implicit in our analyses and calculations is the assumption that metal–ligand complexes do not bind to gills. Otherwise, measured gill metal concentrations may actually represent metal–ligand accumulation on gills. There is reasonable agreement that EDTA and NTA do not pass through cell membranes easily (Jackson and Morgan 1978; Harrison et al. 1984; Pärt and Wikmark 1984; Nor and Cheng 1986; Daly et al. 1990; Block and Pärt 1992), due both to their negative charges and relatively large size. Our present data also gave no indication of ^{14}C-EDTA entry into fish gills (Fig. 8), although entry of ^{14}C-anthracene was easily measured (Fig. 9).

The argument becomes less clear when ligands such as citric acid are used. Citric acid is a small molecule and is a substrate in the tricarboxylic acid cycle. Studies with a number of organisms have shown that Cu and Cd accumulation or toxicity is not reduced as much as expected when small, weak ligands such as citric acid or glycine are used to complex the metals. Competition between ligand and organism for the metal ion, as well as entry (and toxicity) of the small metal–ligand complexes have been discussed in most of these studies, with conclusions favouring ligand competition (Zitko and Carson 1976; Knezovich et al. 1981; Harrison et al. 1984), metal–ligand entry (Guy and Ross Kean 1980; Borgmann and Ralph 1983; Pärt and Wikmark 1984), or a combination of both (Giesy et al. 1977; Poldoski 1979; Laegreid et al. 1983; Nor and Cheng 1986; Daly et al. 1990; Fernandez-Pinas et al. 1991). Block and Pärt (1986) and Block et al. (1991) even showed that ^{109}Cd transfer through perfused gills was enhanced when complexed with xanthate, due to the lipid-soluble nature of the complex formed. Some confusion has undoubtedly occurred because ion selective electrodes, used frequently to measure free metal ions, do not take into account metal that is only weakly bound to a particular ligand and would therefore act as a supply of free metal to another ligand (e.g., gill) with a higher binding constant (Florence 1977; Giesy et al. 1977).

In the absence of any knowledge of the relative strengths of metal–gill and metal–ligand complexes, competition between the gill and ligands for free metal, or uptake of small metal–ligand complexes, are both valid arguments for metal accumulation or toxicity in the presence of weak ligands. However, once it is determined how little free metal is needed for significant metal accumulation on gills (this study), and the strengths of Cd-gill and Cu-gill binding are calculated (Playle et al. 1993), the simpler interpretation of metal accumulation in the presence of weak ligands becomes competition between gills and ligands for free metal ions. For example, the Cd and citrate work of Pärt and Wikmark (1984) suggested that the Cd-citrate complex entered perfused rainbow trout gills, but by their calculations 0.01–0.04 µM Cd (of a total concentration of 9.5 µM) was still free in solution — likely enough free metal to explain their results through entry of free Cd alone. In our experiments, just 0.0001–0.03 µM free Cd was needed to result in significant accumulation of Cd on gills. As another example, this time for freshwater shrimp, the reported toxicity of the Cu-glycine complex and lack of toxicity of the Cu-(glycine)$_2$ complex (Daly et al. 1990) can be explained by the stability constants of the two complexes. When the concentration of glycine is high enough, the Cu-(glycine)$_2$ complex ($\log K = 16.0$; MINEQL$^+$) probably out-competes the freshwater shrimp for Cu, whereas at lower concentrations the weaker Cu-glycine complex may not ($\log K = 8.6$).

Our present work gave no indication of ^{14}C-citrate entry along with Cd (Fig. 10), suggesting that only free metal interacts at the gills. ^{14}C-citrate itself did not enter the gills, even in the presence of DMSO (Fig. 9), a strong surfactant, and there was no indication that ^{14}C-citrate was assimilated and then lost as $^{14}CO_2$. Although metal–ligand entry could be occurring at a slow rate by diffusion, this route of entry does not need to be invoked to explain metal accumulation on gills. Depending on the relative strengths of metal–ligand and metal–gill complexes, and ligand interactions with cations such as H^+ and Ca^{2+}, different concentrations of ligand will be needed to prevent metals from binding to fish gills (Fig. 5, 6, 7). Metal–gill and metal–ligand stability constants, along with ligand concentrations, should therefore be useful for predicting metal accumulation on gills, and hence bioavailability and toxicity.

In summary, measurement of actual metal accumulation on minnow gills has allowed us to determine that Cd binds to gills as well or better than does Cu, probably because of Cd uptake in high affinity Ca channels. This result emphasizes that physiological processes at the gill must be considered when predicting metal accumulation on fish gills. Dissolved organic carbon protects against Cu binding to gills better than it does against Cd binding, which is related to generally greater affinity of Cu to synthetic ligands, and the strong binding of Cd to fish gills. This information, and similar information for other metals, should be useful in predictive models for metal accumulation on gills, and therefore should be useful to predict metal toxicity to fish. Calculation of metal–gill stability constants from our synthetic ligand results, and their use in a model to calculate metal binding to gills of fish exposed to Cu and Cd in natural lake water, is the subject of our companion paper (Playle et al. 1993).

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Copper and Cadmium Binding to Fish Gills: Estimates of Metal–Gill Stability Constants and Modelling of Metal Accumulation

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Playle, R.C., D.G. Dixon, and K. Burnison. 1993. Copper and cadmium binding to fish gills: estimates of metal–gill stability constants and modelling of metal accumulation. Can. J. Fish. Aquat. Sci. 50: 2678–2687.

Fathead minnows (*Pimephales promelas*) were exposed to 17 $\mu g\ Cu \cdot L^{-1}$ or 6 $\mu g\ Cd \cdot L^{-1}$ in synthetic soft water in the presence of competing ligands. Measured gill metal concentrations correlated with free metal ion concentrations, not with total metal. Langmuir isotherms were used to calculate conditional metal–gill equilibrium constants and the number of binding sites for each metal. Log $K_{Cu\text{-}gill}$ was estimated to be 7.4 and the number of Cu binding sites on a set of gills (70 mg, wet weight) was ~2×10^{-8} mol (~30 $nmol \cdot g$ wet $weight^{-1}$). Log $K_{Cd\text{-}gill}$ was ~8.6, and the number of Cd binding sites on minnow gills was ~2×10^{-10} mol (~2 $nmol \cdot g$ wet $weight^{-1}$). Stability constants for H^+ and Ca interactions at metal–gill binding sites and for metal interactions with dissolved organic carbon (DOC) were estimated using these metal–gill constants. All stability constants were entered into the MINEQL$^+$ aquatic chemistry program, to predict metal accumulation on fish gills using metal, DOC, and Ca concentrations, and water pH. Calculated metal accumulation on gills correlated well with measured gill metal concentrations and with LC_{50} values. Our approach of inserting biological data into an aquatic chemistry program is useful for modelling and predicting metal accumulation on gills and therefore toxicity to fish.

Des Têtes-de-boule (*Pimephales promelas*) ont été exposés à du Cu ou du Cd en concentration de 17 $\mu g \cdot L^{-1}$ ou 6 $\mu g \cdot L^{-1}$, respectivement, dans une eau douce synthétique, en présence de ligands concurrents. Les concentrations mesurées de métal sur les branchies étaient en corrélation avec celles des ions métalliques libres, mais pas avec celles du métal total. Des isothermes de Langmuir ont servi à calculer les constantes conditionnelles d'équilibre métal–branchies ainsi que le nombre de sites de fixation dans le cas de chaque métal. Le log $K_{Cu\text{-}branchie}$ a été évalué à 7,4 et le nombre de sites de fixation du Cu sur une paire de branchies (70 mg poids frais) était d'environ 2×10^{-8} mol (~30 $nmol \cdot g$ poids $frais^{-1}$). Le log $K_{Cd\text{-}branchie}$ a été évalué à environ 8,6, et le nombre de sites de fixation de Cd sur les branchies était d'environ 2×10^{-10} mol (~2 $nmol \cdot g$ poids $frais^{-1}$). Les constantes de stabilité des interactions H^+ et Ca sur les sites de fixation des métaux avec le carbone organique dissous (COD), ont été évaluées à partir de ces constantes métaux–branchies. Toutes les constantes de stabilité ont été versées dans le programme de chimie de l'eau MINEOL$^+$ dont on s'est servi pour prévoir l'accumulation des métaux sur les branchies à partir des concentrations des métaux, du COD et du Ca ainsi qu'à partir du pH de l'eau. L'accumulation calculée de métaux sur les branchies était en bonne corrélation avec la concentration de métaux mesurée sur les branchies ainsi qu'avec les valeurs de CL_{50}. Notre méthode d'insertion de données biologiques dans un programe de chimie de l'eau constitue une façon utile de modéliser et de prévoir l'accumulation des métaux sur les branchies, donc de la toxicité de ces métaux pour les poissons.

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It has long been realized that metal-organism stability constants would be useful in interpreting and predicting the toxicity of waterborne metals to aquatic organisms (Biesinger and Christensen 1972; Zitko and Carson 1976). To date, conditional stability constants for Cu have been determined for algae (Xue and Sigg 1990) and for complexing agents released by algae (Van Den Berg et al. 1979; Xue and Sigg 1990) and zooplankton (Fish and Morel 1983). These Cu stability constants fall in the range 10^7–10^{11}, indicating that 50% of the Cu binding sites would be occupied at aqueous Cu concentrations of 10^{-7}–10^{-11} M.

For fish, Reid and McDonald (1991) determined metal–gill stability constants for La, Cu, Cd, and Ca, using excised gills of rainbow trout. Their values, $10^{2.4}$–$10^{3.5}$, were determined using radiolabelled metals in mM metal solutions. These values are low compared to those for algae, most likely because of the relatively high metal concentrations used. The conditional nature of stability constants makes them dependent on the metal concentrations used during their determination: high metal concentrations yield low stability constants because even low affinity sites are able to bind excess metals. At low metal concentrations, only high affinity sites are able to bind the limited amount of metal, yielding higher stability constants.

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Strength of metal binding is not the sole determinant of metal interactions with aquatic organisms. Dissolved organic carbon (DOC), Ca concentration, and pH are important modifiers of metal bioaccumulation and toxicity in freshwater organisms (Yan et al. 1990; Meador 1991; Playle et al. 1992, 1993). Dissolved organic carbon can complex metals, rendering them unavailable to interact with an organism, and calcium competes with metals for binding sites, as does H^+. In addition, water pH determines the proportion of total, unbound metal that is in the cationic form, generally thought to be the toxic species of metals (Morel 1983; Pagenkopf 1983).

The objective of the present study was to determine metal–gill stability constants for low concentrations of Cu and Cd, and to use these constants, plus metal-DOC, Ca-gill, and H-gill constants, in a computer model to predict metal accumulation on fish gills. This approach has been used for algae (Xue and Sigg 1990) but has not yet been applied to fish. Metal accumulation on minnow gills in the presence of complexing ligands such as EDTA were determined in our preceding paper (Playle et al. 1993). We used the lowest concentrations of Cu and Cd (17 and 6 $\mu g \cdot L^{-1}$, respectively) that were feasible to yield measurable metal deposition on fish gills, using graphite furnace atomic absorption spectroscopy. The gill accumulation data plus calculated free metal concentrations in the water were used to determine the metal–gill stability constants. Free metal concentrations were calculated using MINEQL$^+$ (Schecher 1991), an aquatic chemistry computer program based on equilibrium constants. Finally, our estimates of the metal–gill stability constants were assessed by comparing calculated metal accumulation on gills with measured gill metal concentrations of minnows exposed to Cu and Cd in water from a series of Ontario lakes.

Materials and Methods

Ligand Experiments

Detailed descriptions of the ligand experiments are given in the preceding paper (Playle et al. 1993) and the results are summarized in Tables 1 and 2 of the present paper. In brief, acclimated adult fathead minnows (*Pimephales promelas*) were exposed to 17 μg Cu $\cdot L^{-1}$ (0.27 μM) or 6 μg Cd$\cdot L^{-1}$ (0.05 μM) in synthetic soft water (Na, Ca ~50 μM) at pH 6.2, 19°C. Fish were killed at the end of the 2–3-h exposures, their whole gill baskets were removed, weighed, then digested in 1 N H_2SO_4 for 8 h at 80°C. The supernatant of the gill digests was diluted with deionized water, then analyzed for Cu and Cd using graphite furnace atomic absorption spectroscopy. Experiments were run in the presence or absence of freeze-dried dissolved organic carbon (DOC, 0-8 mg$\cdot L^{-1}$) or the synthetic ligands EDTA, nitrilotriacetic acid (NTA), ethylenediamine (EN), and citric, glutamic, oxalic, and salicylic acids (0–2500 μM).

Calculation of Gill Stability Constants

We used Langmuir isotherms to calculate Cu- and Cd-gill stability constants ($K_{Cu\text{-gill}}$, $K_{Cd\text{-gill}}$). In order to make these calculations, estimates of free Cu^{2+} and Cd^{2+} concentrations in the synthetic soft water, with and without added ligands, were made using the MINEQL$^+$ program. Input values were 0.27 μM Cu, 0.05 μM Cd, 70 μM Na, 35 μM Ca, fixed pH 6.2, 19°C, and concentrations of the added ligands (0–2500 μM). Solution ionic strength was calculated by MINEQL$^+$ ($I \sim 1 \times 10^{-4}$), and metal–ligand log K values were supplied by the computer program (Table 1, 2). We ignored changes in water pH near the gills (Playle and Wood 1989; Lin and Randall 1990) because both Cu and Cd exist in the cation form at pH < 7; small pH changes near pH 6 were therefore inconsequential with respect to speciation of these two metals.

Because we had information on Ca and H^+ interactions at Cu and Cd gill-binding sites (Playle et al. 1992, 1993), it was possible to estimate $K_{Ca\text{-gill}}$ and $K_{H\text{-gill}}$ once $K_{Cu\text{-gill}}$ and $K_{Cd\text{-gill}}$ were determined. These estimates were made in the following manner.

Metal–ligand equilibrium constants are defined as the concentration of the metal–ligand complex divided by the product of free metal and free ligand, once the reaction is complete. For example, Cd and gill interactions can be defined as

$$(1) \quad K_{Cd\text{-gill}} = \frac{[\text{Cd-gill}]}{[\text{Cd}] \cdot [\text{gill}]} .$$

Calcium interactions at the same sites on the gill can be defined by

$$(2) \quad K_{Ca\text{-gill}} = \frac{[\text{Ca-gill}]}{[\text{Ca}] \cdot [\text{gill}]} .$$

Rearranging Equation 1, and substituting for [gill] in Equation 2, we get

$$(3) \quad K_{Ca\text{-gill}} = \frac{[\text{Ca-gill}] \cdot [\text{Cd}] \cdot K_{Cd\text{-gill}}}{[\text{Ca}] \cdot [\text{Cd-gill}]} .$$

This equation can be used to calculate $K_{Ca\text{-gill}}$ if we know either how many gill sites, or the proportion of sites, that are occupied by Ca and Cd. If half the gill sites are occupied by Ca, and half by Cd, then Equation 3 simplifies to

$$(4) \quad K_{Ca\text{-gill}} = \frac{[\text{Cd}] \cdot K_{Cd\text{-gill}}}{[\text{Ca}]} .$$

In the situation where the same amount of Cd accumulates on the gills in the presence of Ca as in its absence (i.e., the amount of Ca in solution does not significantly out-compete Cd for the gill binding sites) then

$$(5) \quad K_{Ca\text{-gill}} < \frac{[\text{Cd}] \cdot K_{Cd\text{-gill}}}{[\text{Ca}]} .$$

If the Ca in solution prevents all Cd from accumulating on the gills, then

$$(6) \quad K_{Ca\text{-gill}} > \frac{[\text{Cd}] \cdot K_{Cd\text{-gill}}}{[\text{Ca}]} .$$

Hydrogen ion interactions at metal binding sites can be calculated in the same manner, by substituting H^+ for Ca. Copper

TABLE 1. Gill Cu concentrations (above background) of fathead minnows held for 2–3 h in soft water containing 0.27 µM total Cu, plus ligands. Summarized from Playle et al. (1992, 1993). EN = ethylenediamine.

Ligand (µM)		Free Cu^{2+} (µM; $MINEQL^+$ calculation)	Gill Cu (µmol · g^{-1} wet tissue)	log *K* Cu-ligand (from $MINEQL^+$)
EDTA	0.15	0.112	0.019	18.8
	0.25	0.019	0.000	
	0.30	0.0002	0.000	
NTA	0.25	0.030	0.000	13.1
	2.5	0.0002	0.000	
EN	0.25	0.214	0.036	10.5
	2.5	0.080	0.017	
	25	0.006	0.013	
Citrate	0.25	0.230	0.027	7.3
	2.5	0.122	0.020	
	25	0.007	0.008	
Oxalate	0.25	0.246	0.018	5.1
	2.5	0.199	0.024	
	25	0.044	0.008	
Glutamate	0.25	0.247	0.038	8.3
	2.5	0.210	0.034	
	25	0.101	0.020	
Salicylate	0.25	0.252	0.021	10.6
	2.5	0.249	0.024	
	25	0.225	0.027	
	100	0.176	0.020	
	250	0.127	0.012	
No ligands		0.253	0.026	—
No ligands, no added Cu		0.028	0.000	—

TABLE 2. Gill Cd concentrations (above background) of fathead minnows held for 2–3 h in soft water containing 0.05 µM total Cd, plus ligands. Summarized from Playle et al. (1993). EN = ethylenediamine.

Ligand (µM)		Free Cd^{2+} (nM; $MINEQL^+$ calculation)	Gill Cd (nmol · g^{-1} wet tissue)	log *K* Cd-ligand (from $MINEQL^+$)
EDTA	0.05	14.4	2.0	16.3
	0.10	4.83	2.4	
	0.25	1.41	0.4	
	0.5	0.64	0.0	
	5.0	0.05	0.2	
NTA	0.1	48.7	2.3	9.4
	0.5	46.4	1.6	
EN	0.05	49.3	2.5	5.6
Citrate	0.1	49.1	2.7	5.3
	250	2.60	1.2	
	2500	0.24	0.4	
Oxalate	0.05	49.3	2.1	3.4
Glutamate	0.05	49.3	2.2	4.8
No ligands		49.3	2.0	—
No ligands, no added Cd		0.99	0.0	—

can be substituted for Cd. Implicit in these calculations is the assumption that there is enough metal and time for the gill sites to become saturated. In practice, the metal concentrations and exposure times used in our experiments adequately fulfilled these assumptions (Playle et al. 1992, 1993).

Lakewater Exposures and Toxicity Tests

All stability constants were entered into the $MINEQL^+$ aquatic chemistry program, to calculate metal accumulation on fish gills. To test the model, we wanted to measure metal accumulation on

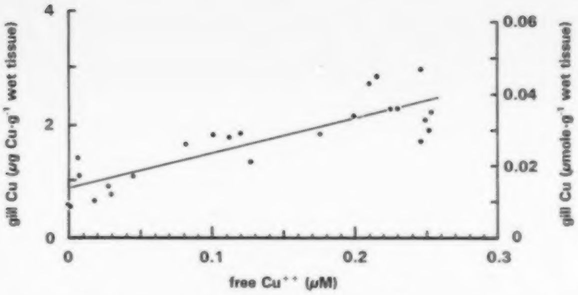

FIG. 1. Plot of measured gill Cu against free Cu^{2+} concentrations. Gill Cu concentrations were from fathead minnows exposed 2–3 h in synthetic soft water with 17 $\mu g\ Cu \cdot L^{-1}$ (0.27 μM) and 0–250 μM of synthetic ligands (data from Playle et al. 1992, 1993). Free Cu^{2+} was calculated using MINEQL⁺ and known concentrations of ligands and ions (Table 1). Gill Cu varied directly with free Cu^{2+} ($r = 0.858$, $P < 0.001$). The equation of the line was $y = 6.23x + 0.86$ (y in $\mu g\ Cu \cdot g\ tissue^{-1}$) or $y = 0.098x + 0.014$ (y in $\mu mol \cdot g\ tissue^{-1}$).

gills when minnows were exposed to 17 $\mu g\ Cu \cdot L^{-1}$ and 6 $\mu g\ Cd \cdot L^{-1}$ in natural soft water, as opposed to synthetic soft water. In addition, we wanted to correlate metal accumulation on gills with metal toxicity.

Water was collected from five lakes NE of Peterborough, Ontario, in June, 1991. The lakes were: Salmon L., Brooks Bay and Sharps Bay of Jack L., Anstruther L., Loucks L., and Wolf L. (all approximately N44°42′, W78°13′). Twenty-L stainless steel containers (Spartenburg Steel Products, Spartenburg, SC) were used to collect the surface water. These containers were then pressurized with N_2 gas and the water was filtered through 1.0 μm glass fibre filters (A/E, 142 mm, Gelman Sciences Inc., Ann Arbor, MI) to remove algae. The filtered water was stored in 40-L stainless steel containers (Simgo Inc., Toronto, ON), brought back to Waterloo, transferred to 4-L polyethylene containers, and stored at 4°C. These water samples were used for combined Cu and Cd metal exposures to adult fathead minnows, conducted in the same manner as described for the synthetic softwater experiments. A synthetic soft water plus a 10-μM (total) mixture of glutamic, citric, and oxalic acids was also used (3.3 μM of each), to mimic a 5 mg $DOC \cdot L^{-1}$ solution (Campbell and Stokes 1985).

Toxicity tests using a geometric series of concentrations of the Cu and Cd mixture were run in the lake waters, using juvenile fathead minnows. These fish were <1 mo old, and had been held in soft water for 1 wk before the experiment. During acclimation they were fed live brine shrimp and fine powdered fish food twice daily. Ninety-six h LC50s were determined using duplicate exposures of five fish per 100-mL solution in un-aerated polyethylene urine cups. Eighteen juveniles (blotted dry) weighed individually yielded an average weight of 1.6 ± 0.5 mg. LC50 values were calculated using a trimmed Spearman–Karber analysis.

Results

Calculation of Metal–Gill Stability Constants

Copper

Previously, we measured Cu accumulation on gills of fathead minnows exposed to 17 $\mu g\ Cu \cdot L^{-1}$ (0.27 μM) in synthetic soft water, in the presence or absence of synthetic ligands (Playle et al. 1992, 1993). Because we knew the water chemistry and the ligand concentrations, we were able to calculate free Cu concentrations using the MINEQL⁺ program. Gill Cu concentrations varied directly with free Cu^{2+}, the major component of free Cu: gill Cu versus free Cu^{2+} gave a straight line (Fig. 1; $r = 0.858$, $P < 0.001$). Gill Cu concentrations did not correlate with total Cu, which was constant at 0.27 μM.

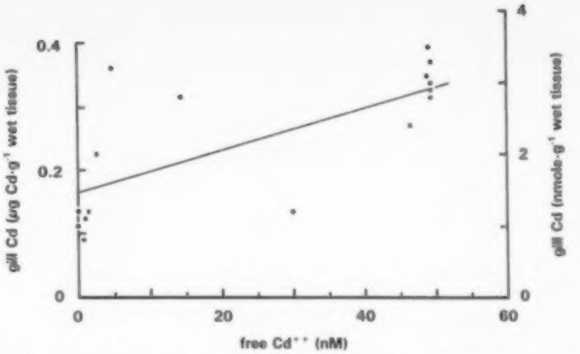

FIG. 2. Plot of measured gill Cd against free Cd^{2+} concentrations. Gill Cd concentrations were from fathead minnows exposed 2–3 h in synthetic soft water with 6 $\mu g\ Cd \cdot L^{-1}$ (0.05 μM) and 0–2500 μM of ligands (data from Playle et al. 1993). Free Cd^{2+} was calculated using MINEQL⁺ and known concentrations of ligands and ions (Table 2). Gill Cd varied directly with free Cd^{2+} ($r = 0.732$, $P < 0.01$). The equation of the line was $y = 0.004x + 0.163$ (y in $\mu g\ Cd \cdot g\ tissue^{-1}$) or $y = 0.031x + 1.451$ (y in $nmol \cdot g\ tissue^{-1}$).

To calculate log $K_{Cu\text{-}gill}$ and the number of Cu binding sites on the gills, a Langmuir plot of Cu adsorption was constructed. Free Cu^{2+} (micromolar) divided by gill Cu (micromoles Cu per gram wet tissue, minus background gill Cu, Table 1) was plotted against free Cu^{2+} (micromolar). The equation for the line was $y = 35.38x + 1.28$. The inverse of the slope of the line is the number of gill binding sites, = 0.03 $\mu mol \cdot g$ wet $tissue^{-1}$. The inverse of the intercept = $K \cdot$ (binding site number), therefore $K = 26\ L \cdot \mu mol^{-1} = 26 \times 10^6\ L \cdot mol^{-1}$, and log $K_{Cu\text{-}gill} = 7.4$. Average total gill weight for the minnows was 0.07 g (wet tissue; Playle et al. 1993), therefore total binding sites for Cu on the gills of our fathead minnows was about 2×10^{-9} mol.

Cadmium

Accumulation of Cd on gills of fathead minnows exposed to 6 $\mu g\ Cd \cdot L^{-1}$ (0.05 μM) in the presence or absence of synthetic ligands (from Playle et al. 1993) was plotted against free Cd^{2+} concentrations calculated using MINEQL⁺ (Fig. 2). Although there were not as many data points as there were for Cu, gill Cd still varied directly with free Cd^{2+} ($r = 0.732$, $P < 0.01$). It was difficult to obtain intermediate values for the curve, because so little Cd was used in the experiments (e.g., 0–50 nM is just one-fifth the scale for Cu in Fig. 1).

The log $K_{Cd\text{-}gill}$ and the number of binding sites for Cd were calculated using a Langmuir plot. Gill Cd concentrations above background (Table 2) were used for these calculations. The Langmuir isotherm, calculated in nmoles, yielded the line $y = 0.44x + 1.06$. The number of gill binding sites was the inverse of the slope, = 2.27 $nmol \cdot g$ wet $tissue^{-1}$. From the inverse of the intercept, $K = 0.42\ L \cdot nmol^{-1} = 0.42 \times 10^9\ L \cdot mol^{-1}$, and log $K_{Cd\text{-}gill} = 8.6$. Average gill weight was 0.07 g wet tissue; thus the number of gill binding sites for Cd was about 2×10^{-10} mol per minnow.

TABLE 3. Input data for the metal-gill interaction model. Initial log K values were as calculated in the text. Final log K values were determined using MINEQL$^+$ to best fit, within the constraints of the initial log K values, calculated gill metal with measured gill metal concentrations for the synthetic soft water system.

Binding sites	Complex	Initial log K	Final log K
Cu-gill	Cu-gillcu	7.4	7.4
2×10^{-9} mol per fish	H$^+$-gillcu	<5.6	5.4
	Ca-gillcu	<3.5	3.4
Cd-gill	Cd-gillcd	8.6	8.6
2×10^{-10} mol per fish	H$^+$-gillcd	>6.1	6.7
	Ca-gillcd	5.0	5.0
DOC	Cu-DOC	8.4–9.4	9.1
$1\ \text{mg} \cdot \text{L}^{-1} = 5 \times 10^{-8}$ mol	Cd-DOC	6.4–8.4	7.4
	H$^+$-DOC	4.0	4.0

Calculation of Ca, H$^+$, and DOC Stability Constants

Calcium and H$^+$ can compete with Cu and Cd for binding sites on gills. By using the log $K_{\text{metal-gill}}$ values calculated above, and data presented in Playle et al. (1992, 1993), the stability constants for Ca and H$^+$ were estimated.

Copper accumulation on minnow gills was not affected at pH 4.8 (15.8 μM H$^+$) compared to pH 6.3 (0.5 μM H$^+$; Playle et al. 1992). Inserting $K_{\text{Cu-gill}} = 10^{7.4}$, free Cu = 2.53×10^{-7} M (MINEQL$^+$ calculation), and H$^+ = 15.8 \times 10^{-6}$ M into Equation 5 yields log $K_{\text{H-gill}} < 5.6$. Copper accumulation was not affected by 2 000 μM Ca (Playle et al. 1992): using Equation 5, Cu = 2.53×10^{-7} M, Ca = 2×10^{-3} M, and $K_{\text{Cu-gill}} = 10^{7.4}$, yields log $K_{\text{Ca-gill}} < 3.5$.

Cadmium accumulation on the gills was reduced at pH 4.8 (Playle et al. 1993). Inserting $K_{\text{Cd-gill}} = 10^{8.6}$, free Cd = 4.93×10^{-8} M (MINEQL$^+$ calculation), and H$^+$ = 15.8×10^{-6} M into Equation 6 yields log $K_{\text{H-gill}} > 6.1$. For Ca, 95 μM did not reduce Cd accumulation on gills, whereas 1 050 μM Ca did (Playle et al. 1993). Using Equations 5 and 6, respectively, log $K_{\text{Ca-gill}} < 5.3$, but >4.3, the mean of which is log $K_{\text{Ca-gill}} = 5.0$.

Five to 6 mg DOC $\cdot$ L^{-1} prevented Cu accumulation on gills, by complexing all the 0.27 μM Cu in solution (Playle et al. 1993). Thus, there were at least 0.05 μmol binding sites per mg DOC $\cdot$ L^{-1}. These metal binding sites must have had a higher affinity for Cu than did the Cu binding sites on the gill, or the DOC would not have been able to prevent Cu accumulation on the gills. The log $K_{\text{Cu-DOC}}$ value for the 0.05 μmol of high affinity binding sites per mg $\cdot$ L^{-1} DOC must therefore be about 1–2 log units higher than the log $K_{\text{Cu-gill}} = 7.4$ value. Our initial estimate of the stability constant was log $K_{\text{Cu-DOC}} = 8.4$–9.4, which is in the mid-range of published values (Van Den Berg and Kramer 1979; Morel 1983). Note that direct attack of the Cu-gill complex by DOC is unlikely (adjunctive pathway of ligand exchange; see Hering and Morel 1990). More likely is the disjunctive pathway, that first involves dissociation of Cu from the Cu-gill complex, the free Cu then being able to react with DOC in solution.

Cadmium has consistently been reported to bind <10× as well to DOC than does Cu (Alberts and Giesy 1983; Tuschall and Brezonik 1983). Thus, an initial estimate of the log $K_{\text{Cd-DOC}}$ value of 10–100× less than for Cu would be 6.4–8.4. The log K for humic substances is 3 to 4 (DeWit et al. 1991), therefore we set log $K_{\text{H-DOC}} = 4$. We had no information on the interactions of Ca with DOC.

Model Development

Our purpose in determining metal–gill and other stability constants was to use these values in a computer model to predict metal accumulation on fish gills, and thereby predict metal toxicity. In essence, bringing biology into an aquatic chemistry program. The model we chose to use was MINEQL$^+$ (Schecher 1991), an aquatic chemistry program based on log K stability constants.

Our initial estimates of log K stability constants and the number of metal binding sites are summarized in Table 3. To input these data into the MINEQL$^+$ program, three null components were defined (gillcu, gillcd, and DOC). We simulated two different sites for Cu and Cd binding on the gills (gillcu, gillcd) on the basis of our previous results (Playle et al. 1993) that indicated, for the low metal concentrations we used, no competition between Cu and Cd for gill binding sites. Metal, Ca, and H$^+$ interactions with gillcu, gillcd, and DOC were defined in the program as 1:1 reactions (Table 3).

We used Na = 70 μM, Ca = 35 μM, Cd = 0.05 μM, Cu = 0.27 μM, fixed pH 6.2, and 19°C for water chemistry parameters of the synthetic soft water. For log K values for which we just had upper or lower values, or a range, we chose final log K values after optimizing to best fit the observed data in Playle et al. (1992, 1993). In essence, the model was able to mimic the greater effect of H$^+$ and Ca on Cd binding to gills, and the greater effect of DOC on Cu binding to gills. Of note in Table 3 are the final log $K_{\text{metal-DOC}}$ values, that we optimized to log $K_{\text{Cu-DOC}} = 9.1$ (in the model, Cu binds to DOC 50× better than it binds to the gill), and log $K_{\text{Cd-DOC}} = 7.4$ (in the model, Cd binds to DOC 50× less well than does Cu, and Cd binds to DOC 16× less well than Cd binds to gills). We chose 1 mg $\cdot$ L^{-1} DOC = 0.05 μmole binding sites, to allow enough binding sites for all the Cu and Cd in solution at 6 mg DOC $\cdot$ L^{-1} (e.g., no competition by the metals for the sites). In general, as long as a ligand is in excess relative to metals in solution, metals do not compete with each other (Morel 1983).

Metal Accumulation and Toxicity to Fish Exposed to Cu and Cd in Soft Lake Water

Of the natural lake waters that we used in the Cu and Cd exposures, Salmon L., and Brooks and Sharps bays contained more Ca, more DOC, and had higher pH than did Anstruther, Loucks, and Wolf lakes (Table 4). The synthetic soft water plus a 10 μM (total) addition of glutamic, citric, and oxalic acids had water chemistry similar to that of synthetic soft water without

TABLE 4. Lake water and synthetic soft water characteristics, and juvenile fathead minnow 96 h LC50s. Water characteristics were measured on water used for the metal deposition and LC50 experiments, except for conductivity, which was from a 1988 survey. SSW = synthetic soft water; + ligands = plus 10 μM (total) glutamic, citric, and oxalic acids. DOC = $\mathrm{mg \cdot L^{-1}}$, Ca and Na = μM, conductivity (Cond.) = $\mu\mathrm{mho \cdot cm^{-1}}$, and all metals = $\mu\mathrm{g \cdot L^{-1}}$. Mean ± SEM (*n*) if $n > 2$. For LC50, values in parentheses = 95% CI.

											96 h LC50	
Water	pH	DOC	Ca	Na	Cond.	Cu	Cd	Al	Fe	Zn	Cu	Cd
Salmon Lake	8.05±0.01 (12)	6.4±0.1 (3)	695±20 (4)	34±6 (4)	85	2.3	0.1	10.7	<50	12.0	>126	>32
Brooks Bay	7.80±0.01 (12)	9.8 (2)	445±10 (4)	76±9 (4)	—	3.1	0.1	12.8	<50	4.0	102 (78–133)	25.3 (19.1–33.4)
Sharps Bay	7.90±0.02 (12)	7.9 (2)	590±20 (4)	90±32 (4)	78	6.6	<0.1	6.5	<50	2.5	115 (85–156)	27.8 (19.8–39.0)
Anstruther Lake	7.10±0.03 (12)	7.2±0.6 (3)	128±2 (4)	39±5 (4)	24	4.1	0.1	12.8	<50	1.5	50.0 (31.9–54.4)	10.4 (7.8–14.0)
Loucks Lake	7.04±0.01 (12)	6.6 (2)	92±2 (4)	37±4 (4)	22	1.4	0.3	13.3	<50	4.2	27.8 (18.4–42.0)	6.8 (4.2–11.1)
Wolf Lake	6.91±0.03 (12)	6.0±0.2 (3)	94±1 (5)	48±12 (4)	19	0.8	0.3	9.6	<50	1.6	31.2 (21.9–44.6)	6.9 (4.0–11.9)
SSW + ligands	6.60±0.03 (8)	1.8 (2)	36±1 (3)	87±23 (3)	—	—	—	—	—	—	10.3 (6.8–15.6)	1.1 (0.4–3.0)
SSW	6.69±0.01 (20)	2.5 (2)	37±1 (11)	91±11 (11)	—	2.1±0.3 (32)	<0.1 (33)	4.4	<50	2.8±0.5 (10)	9.4 (7.6–11.5)	1.0 (0.6–1.7)

added ligands. Minnow exposures in both these synthetic soft waters were at pH ~6.7, to match the pH of the softer of the natural waters.

Copper and Cd 96-h LC50 values for juvenile fathead minnows exposed in the various waters are given in Table 4. In general, LC50s for the Cu and Cd mixtures were lowest (metals were most toxic) in softer water. Metals were most toxic in the synthetic soft water, and were only slightly less toxic in the presence of the 10 μM ligand mixture. Metals were least toxic in water from Salmon L., with too few mortalities to accurately determine the LC50 (Table 4).

Cadmium accumulation on gills of adult minnows exposed to 6 $\mu\mathrm{g\ Cd \cdot L^{-1}}$ and 17 $\mu\mathrm{g\ Cu \cdot L^{-1}}$ was highest in Wolf L. water (significantly above background, and not significantly different from metal-exposed fish in synthetic soft water alone; Fig. 3), and lowest in Salmon L. water (not significantly above background). Fish exposed to the lake waters without added metals did not have gill Cd concentrations significantly above background (0.18 ± 0.02 $\mu\mathrm{g\ Cd \cdot g}$ wet $\mathrm{tissue^{-1}}$; ±95% CI, $n = 17$). Fish exposed to Cd and Cu in synthetic soft water plus 10 μM (total) glutamic, citric, and oxalic acids had 0.64 ± 0.23 (6) $\mu\mathrm{g\ Cd \cdot g}$ wet $\mathrm{tissue^{-1}}$, and fish exposed to the metals in synthetic soft water without added ligands had gill Cd concentrations of 0.54 ± 0.05 (9) $\mu\mathrm{g\ Cd \cdot g}$. These exposures were run at pH 6.6 and 6.7, respectively; their gill Cd concentrations were not significantly different from those of fish exposed in synthetic soft water at pH 6.2.

Copper accumulation on gills of adult minnows was never above background in fish held in lake waters supplemented with Cu and Cd (Fig. 4), probably because DOC exceeded 5 $\mathrm{mg \cdot L^{-1}}$ in all lake waters (see Playle et al. 1993). In lake water without added metals, gill Cu was 0.80 ± 0.08 (17), also not significantly above background. Minnows exposed to Cu and Cd in synthetic soft water plus the 10 μM ligand mixture (pH 6.6) had gill Cu concentrations of 2.58 ± 0.18 (6) $\mu\mathrm{g\ Cd \cdot g}$ wet $\mathrm{tissue^{-1}}$, while fish exposed to the metals in synthetic soft water without added ligands (pH 6.7) had gill Cu concentrations of 2.16 ± 0.30 (9) $\mu\mathrm{g\ Cu \cdot g^{-1}}$. Neither concentration was significantly different from gills of minnows exposed to Cu in synthetic soft water at pH 6.2.

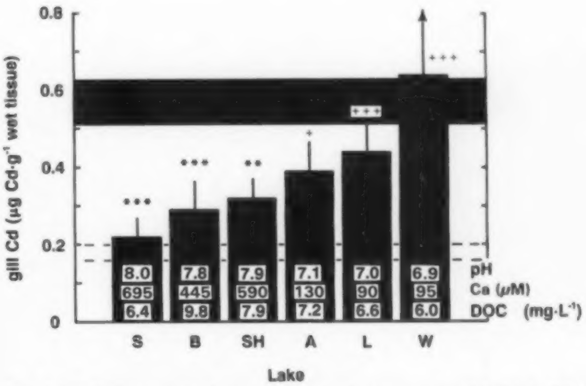

FIG. 3. Gill Cd concentrations of fathead minnows exposed 2–3 h in Cd- and Cu-supplemented lake water. Cadmium deposition on gills was highest in the softest water. S = Salmon L., B = Brooks Bay, SH = Sharps Bay, A = Anstruther L., L = Loucks L., and W = Wolf L. Vertical lines on bars = 95% CI; $n = 6$ for each bar. Dashed horizontal lines represent the 95% CI of gill Cd for minnows held in synthetic soft water in the absence of added metals ($n = 38$). The grey horizontal band represents the 95% CI of gill Cd for fish held 2–3 h in synthetic soft water with 6 $\mu\mathrm{g\ Cd \cdot L^{-1}}$ ($n = 45$). *, **, *** = significantly below metal-exposed gills (grey band), and +, ++, +++ = significantly above background gill metal concentration (dashed lines) for $P < 0.05$, <0.01, <0.001, respectively.

Model Testing

Final values for the log *K* stability constants (Table 3) were used to assess the usefulness of the model in predicting gill metal concentrations and metal toxicity. In the model we used the

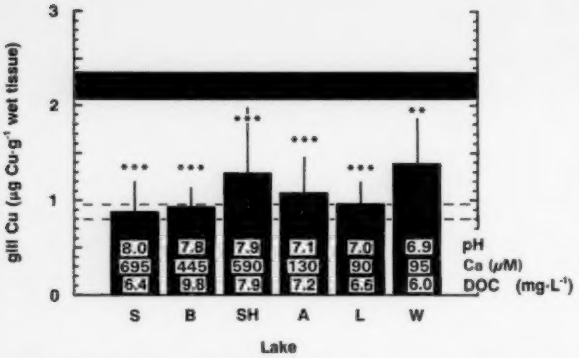

FIG. 4. Gill Cu concentrations of fathead minnows exposed 2–3 h in Cd- and Cu-supplemented lake water. There was no significant accumulation of Cu on the gills, likely because DOC was ≥ 6 mg · L^{-1} in each lake water. Dashed horizontal lines represent the 95% CI of gill Cu for minnows held in synthetic soft water alone ($n = 38$). The grey horizontal band represents the 95% CI of gill Cu for fish held 2–3 h in synthetic soft water with 17 µg Cu · L^{-1} ($n = 45$). Lake names and other details are given in caption of Fig. 3.

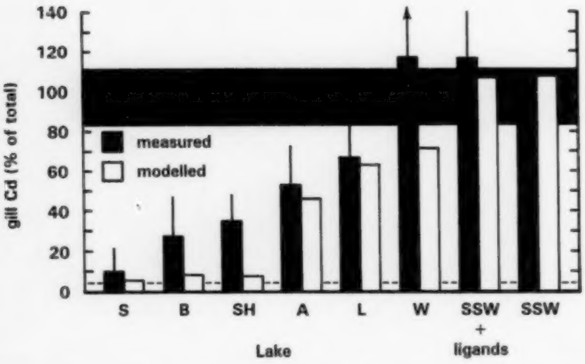

FIG. 5. Measured and modelled gill Cd, for fathead minnows exposed 2–3 h to 6 µg Cd · L^{-1} and 17 µg Cu · L^{-1} in natural soft waters and synthetic soft water (SSW) with or without 10 µM added ligands (at pH 6.7). Lakes are defined in the caption of Fig. 3. Gill Cd is expressed as a percentage of the mean gill Cd concentration (100%) for fish held in synthetic soft water plus Cd at pH 6.2 (grey bar, ±95% CI). Background gill Cd = 0% (+95% CI, dashed line). Calculated gill Cd correlated well with measured gill Cd (see text for details). Measured gill Cd values are given with their 95% CI.

DOC, Ca, and Na values (molar), and pH (fixed) for the eight waters listed in Table 4. The model mimicked a system open to atmospheric CO_2. Although we did not measure CO_3^{2-} in the lake waters, for the model we assumed its concentration was equal to that of Ca (Table 4), which yields approximately the same values as if the more rigorous Henderson-Hasselbalch equation were used to calculate HCO_3^-. For the comparison of measured and calculated gill Cd and Cu, measured values from Fig. 3 and 4 were converted to percentages, with background gill metal = 0%, and gill metal concentrations of fish exposed to metals in synthetic soft water (at pH 6.2) = 100%. The calculated gill metal concentration in synthetic soft water (pH 6.2, assuming background DOC = 1 mg · L^{-1}) was used as the model 100% value.

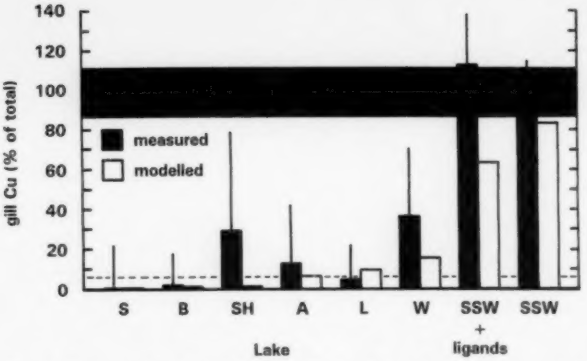

FIG. 6. Measured and modelled gill Cu for fathead minnows exposed 2–3 h to 17 µg Cu · L^{-1} and 6 µg Cd · L^{-1} in natural soft waters and in synthetic soft water (SSW) with or without added ligands. Lakes are defined in the caption of Fig. 3. Gill Cu is expressed as a percentage of the mean gill Cu concentration (100%) for fish held in synthetic soft water plus Cu (grey bars). Background gill Cu = 0% (dashed line). Calculated and measured gill Cu correlated well (see text for details).

Modelled gill Cd agreed well with our measured values (Fig. 5), with the largest discrepancy for Sharps Bay. The correlation coefficient between measured and modelled gill Cd was 0.9008 ($P < 0.01$). Measured gill Cd versus the juvenile fathead minnow LC50s for Cd (Table 4) yielded a correlation coefficient of −0.897 ($P < 0.01$). Modelled gill Cd versus the LC50 values yielded a better correlation ($r = -0.960$; $P < 0.001$). These data indicate that model-calculated gill Cd concentrations could reasonably be used to predict acute Cd toxicity to fish.

Measured and modelled gill Cu also agreed well (Fig. 6; $r = 0.926$, $P < 0.001$), in spite of the fact that none of the gill Cu concentrations from the natural lakewaters was above background (Fig. 4). Measured gill Cu versus the log of the juvenile fathead minnow LC50s for Cu yielded $r = -0.830$ ($P < 0.05$; gill Cu vs. linear LC50s was not significant, $r = -0.662$, $P > 0.05$). Modelled gill Cu had a slightly better correlation with the log LC50s ($r = -0.895$, $P < 0.01$; the correlation with linear LC50s was $r = -0.727$, $P < 0.05$). As was the case with Cd, modelled gill Cu concentration was a good indicator of acute Cu toxicity.

Discussion

In the present study, we have better defined interactions of Cu and Cd with fish gills, as influenced by dissolved organic carbon (DOC), pH, and Ca. We used Langmuir isotherms to estimate conditional metal–gill stability constants. These were $\log K_{Cu\text{-}gill} = 7.4$ and $\log K_{Cd\text{-}gill} = 8.6$. There were about 10× more of the weaker Cu sites on the gills (~2×10^{-9} mol per fish) than Cd sites (~2×10^{-10} mol per fish).

Interactions of Cu and Cd with the gill and with DOC, and competing reactions with Ca and H^+, are illustrated conceptually in Fig. 7, along with their log K values. Cadmium binds to the gills better than does Cu, and is more affected by competition from Ca and H^+. These interactions are likely a consequence of active Cd uptake through high affinity Ca channels in addition to general surface binding. Cadmium reduces Ca uptake, mainly through effects on basolateral Ca^{2+}–ATPase activity (Verbost et al. 1989). The data of Reid and McDonald (1988) support the existence of higher affinity binding sites for Cd compared to Cu. Calcium competition reduces Cd uptake at gills, and therefore the subsequent distribution to the rest of the fish body (Wicklund and Runn 1988). Transfer of metals through the basolateral membrane is probably by diffusion (Verbost et al. 1989).

Copper, on the other hand, appears to bind to weaker sites on the gills, and possibly also enters through ion channels (Fig. 7), but binds more strongly to DOC than does Cd (log $K_{\text{Cu-DOC}} = 9.1$; log $K_{\text{Cd-DOC}} = 7.4$). The binding order for DOC reflects relatively strong binding of Cu to ligands. The effect of low concentrations of Cu at the gills is an inhibition of Na^+ influx, mainly through effects on Na^+–K^+–ATPase (Laurén and McDonald 1987). Relatively more inhibition of this effect was seen by carbonate complexation than by Ca (Laurén and McDonald 1986), which agrees with our results of less protective effects of Ca on Cu accumulation, compared to effects on gill Cd (Playle et al. 1992, 1993). Note that in the conceptual model illustrated in Fig. 7 the gill mucus layer is ignored. Gill mucus probably binds metals and slows metal access to the gill (Pärt and Lock 1983). However, once a metal exceeds the complexing and sloughing ability of mucus, the metal will become available to react at the gill epihelium; this is the situation illustrated in Fig. 7.

There are few log $K_{\text{metal-gill}}$ values with which to compare our values. Reid and McDonald (1991) found log K values of about 3.5, 3.0, 3.0, and 2.4 for La, Cd, Ca, and Cu, respectively. These values are low, probably because they used mM concentrations of metals in their experiments instead of environmental, sub-micromolar concentrations. Log K values are sensitive to the metal concentration at which the experiments are run, and it is difficult to extrapolate from a stability constant obtained at high metal:ligand ratio to a stability constant for a low metal:ligand ratio (Perdue and Lytle 1983). However, Reid and McDonald (1991) found that Cd bound to gills better than did Cu, which agrees with our results. Xue and Sigg (1990) determined log $K_{\text{Cu-algae}} = 8.8$ (at pH 6.0), which is ~10× higher than ours for fish at about the same pH. These workers were successful in modelling Cu binding to algae using a computer program based on log K values, their input log $K_{\text{Cu-algae}}$ values, and the number of binding sites (2 μmol · g dry algae^{-1}) they determined for algae.

We inserted our metal–gill, H^+-gill, Ca-gill, and metal-DOC stability constants (Fig. 7; Table 3) into MINEQL$^+$ (Schecher 1991). The model adequately reflected measured metal accumulation on the gills (Fig. 5, 6). In addition to Cu and Cd bound to gills, the MINEQL$^+$ output included Ca and H^+ bound to gill sites (an indication of competition for the sites), unoccupied gill sites, free metal in solution (an indication of the pool of metal available to bind at the sites), and metal bound to DOC (representing the extent of metal complexation). This simple model considered only 1:1 metal-ligand interactions, and ignored Ca interactions at metal binding sites on DOC. At the low metal concentrations we used, 1:1 binding would be expected: Cabaniss and Shuman (1988a) showed that Cu-DOC interactions were >90% 1:1 binding. Not incorporating Ca-DOC interactions probably would have had little effect on the model, because both Cu and Cd would bind much more strongly to DOC than would Ca (Kerndorff and Schnitzer 1980; Cabaniss and Shuman 1988a; Daly et al. 1990). It should be noted that our stability constants represent average conditional stability constants, akin to continuous multiligand binding models (e.g., Perdue and Lytle 1983; Grimm et al. 1991) or "quasiparticle" models (Sposito 1981).

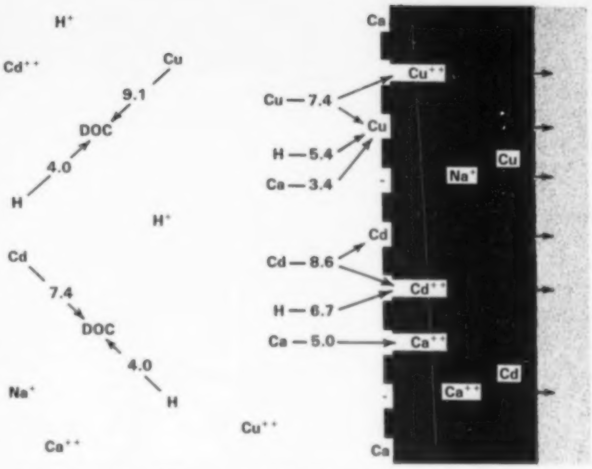

FIG. 7. Conceptual illustration of Cu, Cd, Ca, and H^+ interactions at fish gills and with dissolved organic carbon (DOC). The numbers are the log K conditional stability constants for the interactions. In water (left), Cu and Cd may become complexed by DOC, and also face competition from Ca and H^+ for negative binding sites on gill surfaces. As well as binding to the general gill surface, Cu may enter the gill through low affinity channels. Copper disrupts Na^+ uptake at the basolateral Na pump. The stability constant for Cd is higher than for Cu: if surface binding was solely responsible for metal reactions at the gills, Cd would be expected to bind less strongly than Cd. Uptake of Cd through high affinity Ca channels is likely the explanation of the higher-than-expected $K_{\text{Cd-gill}}$. Also illustrated is the inhibition of the basolateral Ca pump by Cd.

Stability constants change with solution chemistry (pH, ionic strength) as well as with metal and ligand concentration (Perdue and Lytle 1983; Cabaniss and Shuman 1988a). Van Den Berg and Kramer (1979) and Morel (1983) determined that there is about a one log unit decrease in stability constants as pH decreases by one unit. However, we used the approach of Grimm et al. (1991) and Cabaniss and Shuman (1988a) where changes in stability constants are considered to be due solely to H^+ competition. That is, the use of one metal–gill stability constant, with another H^+ stability constant for competition, as opposed to different metal–gill constants for each pH. In spite of the qualifications and simplifications used in the model, relative amounts of Cu and Cd deposition on the gills were reasonably well predicted (Fig. 5, 6). Copper was complexed better by DOC than was Cd, and Cd binding to the gills was affected more by Ca than was Cu.

We calculated approximately 0.05 μmol metal binding sites per mg DOC. Reported number of Cu binding sites for DOC vary from about 10 μmol per mg DOC (Cabaniss and Shuman 1988a) to 0.2 μmol per mg DOC (Hering and Morel 1990). Our low number of binding sites is indicative of the low metal concentrations we used in our experiments. The sites were relatively strong metal binding sites (log $K_{\text{Cu-DOC}} = 9.1$, log $K_{\text{Cd-DOC}} = 7.4$), which may represent metals binding with carboxyl groups (Taga et al. 1991). Sunda and Hanson (1979) determined log $K_{\text{Cu-DOC}} =$ 9.0 for river water at pH 5.95, very similar to our Cu result. Hering and Morel (1990) used 0.05 μM Cu concentrations in their experiments, and found slightly stronger Cu binding to humic acid (log $K_{\text{Cu-humic acid}} = 10.1$). Holm and Curtiss (1990) used 0.05, 0.2, and 10 μM Cu in their experiments, and found log $K = 10.1$, 8.5, and 5.5, respectively, for natural organic matter in ground water, demonstrating the dependence of stability constants on the metal concentration used in their determination. Grimm et al. (1991) determined (mean) log $K_{\text{Cu-DOC}} = 4.2$–4.9, a much lower stability constant than those listed above, but they used experimental Cu concentrations ≥ 5 μM. Holm and Curtiss (1990) determined that complexes with natural organic matter should dominate Cu speciation at the low Cu concentrations they used. In agreement, our measured and modelled gill Cu values (Fig. 6) were mainly influenced by DOC concentration.

Cadmium has been shown to bind less well than Cu to DOC and fulvic and humic acids (e.g., Florence 1977; Kerndorff and Schnitzer 1980; Alberts and Giesy 1983; Lund et al. 1990; Sahu and Banerjee 1990). This binding order is related to general metal-ligand binding strength. If Cu binds better to DOC and humic acids, then Cu toxicity would be expected to decrease in response to organic material more than does Cd toxicity (e.g., Winner 1985, 1986 for Cu, Cd toxicity to *Daphnia pulex*). In our work, freeze-dried DOC protected against Cu deposition on minnow gills at concentrations ≥ 4.8 $\mathrm{mg \cdot L^{-1}}$ (Playle et al. 1993), and insignificant Cu accumulation was observed in natural lake waters containing ≥ 6 mg DOC $\cdot \mathrm{L^{-1}}$ (Fig. 6), whereas Cd still bound to fish gills in the presence of ≥ 6 mg DOC $\cdot \mathrm{L^{-1}}$ (Fig. 5). Because of these binding results, we suggest that toxicity of the metal mixtures in soft waters (Table 4) was due mainly to Cd.

In the model we ignored differences in DOC source or composition, because concentration of DOC adequately determined gill Cu concentrations (Playle et al. 1993). Although DOC from different sources can bind or detoxify metals to varying degrees (e.g., Nor and Cheng 1986; Sahu and Banerjee 1990), DOC from similar sources (i.e. same size of watershed and vegetation types) generally binds metals in a similar manner (Oliver et al. 1983; Cabaniss and Shuman 1988b). Source of DOC may not be as important as pH, alkalinity, or ionic strength in determining metal binding to DOC (Cabaniss and Shuman 1988b), and these workers suggested that experimental effort should be expended in defining these effects, not on documenting variations in DOC binding properties with season or location.

Synthetic soft water plus 10 μM (total) citric, glutamic, and oxalic acids did not reduce Cu or Cd accumulation on fathead minnow gills, nor did it alter metal toxicity (Table 4). This mixture was meant to be a synthetic DOC analogue, similar to but simplified from those used by Sposito (1981) and by Campbell and Stokes (1985). Campbell and Stokes (1985) found that a 22 μM DOC analogue complexed ~70% of a 6 μg Cu $\cdot \mathrm{L^{-1}}$ solution (pH 6). With 3× that amount of Cu in our experiments, and half the ligand concentration, only about 12% of the Cu would be expected to be complexed, not enough to reduce Cu accumulation on gills. However, the model did calculate some reduction in free Cu (to about 60% of total; Fig. 6). It is not surprising that the 10 μM (total) ligand solution did not reduce Cu or Cd bound to gills or reduce metal toxicity, and it does not adequately reflect 5 mg DOC $\cdot \mathrm{L^{-1}}$ in our system.

The model should be expanded to include other metals. It may be possible to calculate stability constants from previously published ligand work, but some metal–gill stability constants have already been published. For example, Wilkinson et al. (1990) used salmonid data to estimate $\log K_{\text{Al-gill}} \sim 6.5$. Conditional stability constants for organic material and Al are available: $\log K_{\text{Al-DOC}} = 6.2$ (Urban et al. 1990), $\log K_{\text{Al-fulvic acid}} = 7.8$ (Shuman 1992). Constants are also available for other metals such as Mn ($\log K_{\text{Mn-DOC}} = 3.8$; Urban et al. 1990), Fe ($\log \mathrm{K}_{\text{Fe-DOC}} = 9.4$; Urban et al. 1990), and Co ($\log K_{\text{Co-humic acid}} =$ 5-6; Van Loon et al. 1992). Weaker complexing metals such as Ni and Zn would likely bind to DOC about 10× less well than does Cu (Morel 1983). Lin and Benjamin (1992) indicated some of the confounding effects of ligand addition to a multiple-metal system. A ligand may complex a metal that is otherwise bound to a (gill) binding site, which would free that site (e.g., increase free gill sites) to which another metal may bind. Models such as ours will be useful to predict complex interactions of this type.

In an expanded model, competition for gill binding sites by Ca and $\mathrm{H^+}$ need to be considered for each metal using appropriate log K values. Metals will be less toxic in hard water, and very acidic conditions will keep metals off gills. Of course, $\mathrm{H^+}$ itself can be disruptive to gills. Simulations using our model indicated that $\mathrm{H^+}$ would protect against metal binding at gills before it would displace metal from DOC. By the time enough metal was displaced from DOC for the metal to interact at the gills ($\mathrm{pH} < 4$), $\mathrm{H^+}$ itself would likely become toxic. This point illustrates the usefulness of the model in integrating multiple effects such as speciation, competition, and complexation, a situation where models relying on correlation (Yan et al. 1990; Meador 1991) are often inappropriate.

Output from the present model is toxicant bound to the gills, the target organ, which then must be translated into toxicity to the fish. This last step requires the most study. Together, the model output would give an indication of all types of competition for gill binding sites, of which Ca interactions can be considered beneficial, and most others as potentially toxic. In our lake water experiments, measured and calculated gill Cu and Cd concentrations did correlate with toxicity, but it is not known how much metal accumulation is necessary to affect fish survival on a long-term basis. Presumably, a constant accumulation (gill "dose") will be toxic, such as was found for whole-body Cd accumulation in *Hyalella azteca* (Borgmann et al. 1991).

In summary, measurement of metal deposition on minnow gills has allowed us to estimate conditional stability constants of Cu and Cd binding to gills, the number of metal binding sites on the gills, plus interactions with Ca, $\mathrm{H^+}$, and DOC. This information, and similar information for other metals, will be useful in predictive models for metal accumulation on gills, and will therefore be useful to predict metal toxicity to fish.

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Pelagic Food Web Structure in Acidic Adirondack Mountain, New York, Lakes of Varying Humic Content

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Havens, K.E. 1993. Pelagic food web structure in acidic Adirondack Mountain, New York, lakes of varying humic content. Can. J. Fish. Aquat. Sci. 50: 2688–2691.

Pelagic food web models were constructed for 28 acidic ($pH < 5.0$) Adirondack Mountain lakes, and multiple regression analyses were done to quantify relationships between abiotic variables (DOC, monomeric Al, total P, and H^+ concentrations and lake area) and three food web attributes (number of species, number of links, and links per species) Regression models contained log DOC and H^+ as significant predictors of food web structure. The models explained over 35% of the variation in species, links and links per species; most of the variation was explained by log DOC. Species, links and links per species increased as DOC increased from <2 to roughly $4\ mg \cdot L^{-1}$. At higher DOC levels, the food web attributes reached plateaus at 35 species, 120 links, and 4 links per species. The results support the view that in acidic lakes, DOC ameliorates the toxic effects of Al, so that above a saturating DOC level of approximately $4\ mg \cdot L^{-1}$, Al-sensitive biota can exist in acid waters. Hence, food webs of high DOC acid lakes are larger and more complex than their low DOC counterparts.

On a élaboré des modèles de chaînes trophiques pélagiques de 28 lacs acides des Adirondacks ($pH < 5,0$); on a fait des analyses par régression multiple afin de quantifier les rapports entre les variables abiotiques (concentrations du COD, de l'Al ionique, du P total et de H^+, ainsi que la superficie de chaque lac) et trois propriétés des chaînes trophiques (nombre d'espèces, nombre de liens et liens par espèce). Les modèles par régression avaient le log COD et H^+ comme indicateurs significatifs de la structure du réseau trophique. Les modèles permettaient d'expliquer plus de 35 % des écarts dans le nombre d'espèces, et de liens et de liens par espèce; la majeure partie des écarts était expliquée par le log COD. Le nombre d'espèces, de liens et de liens par espèce augmente à mesure que le COD passe de <2 à environ $4\ mg \cdot L^{-1}$. À une concentration de COD supérieure, les propriétés du réseau trophique atteignent un plateau à 35 espèces, 120 liens et 4 liens par espèce. Le résultats obtenus confirment que dans les lacs acides, le COD atténue les effets toxiques de l'Al de sorte qu'au-delà d'un niveau de saturation en COD d'environ $4\ mg \cdot L^{-1}$, les biotes sensibles à l'Al peuvent exister dans des eaux acides. Il en découle que les réseaux trophiques des lacs acides à forte concentration en COD sont plus gros et plus complexes que ceux des lacs à faible concentration en COD.

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Acidic lakes number in the thousands in glaciated North America, and in some regions they account for a large fraction of the surface waters. In the Adirondack Mountains of New York State, a region severely affected by acid deposition, Sutherland (1989) found that over 50% of surveyed lakes had pH below 5.0. Many studies have been done to characterize biotic responses to acidification. Population declines and species extinctions have been documented at all trophic levels (Baker et al. 1990), and it has been shown that pelagic food webs in acidic lakes are greatly simplified relative to their higher pH counterparts (Havens 1993a). With declining pH, there are fewer species, fewer predator–prey links, and fewer links per species. Acidic lake food webs are also more variable (Locke 1992; Havens 1993a), and it has been suggested that they may reflect differing levels of humic material and toxic aluminum species in the water (Havens 1993a). Aluminum concentrations typically become very high in precipitation-acidified lakes (Linthurst et al. 1986; Spry and Wiener 1991), because acid rain water leaches Al from clay soils, and because Al becomes increasingly soluble in lake water at low pH. Aluminum is toxic to fish, invertebrates and algae (Baker and Schofield 1980; Havens 1993a), but its toxicity may be reduced if dissolved organic carbon (DOC) compounds are present in the water (Baker and Schofield 1980; McCahon and Pascoe 1989). Previous studies in the Adirondacks showed that Al and DOC levels affect the composition of both phytoplankton and zooplankton assemblages (Siegfried et al. 1989a, 1989b; Sutherland et al. 1989). No such investigation has been done for the pelagic food web as a whole.

In the present study, I tested the hypothesis that humic content is a major determinant of pelagic food web size and complexity in acidic Adirondack Mountain lakes. The approach was to construct pelagic food web models for 28 acidic lakes, and then quantify relationships between food web attributes and several abiotic variables, including DOC. The food webs described herein contain phytoplankton, zooplankton, invertebrate predators and fish. They lack certain components (heterotrophic protists and bacteria), but represent the most complete taxonomic coverage to date in any such study of acidic lakes.

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Methods

Data Set

Species lists and physical and chemical data were obtained from Sutherland (1989), who also provided methodological details. The 50 lakes surveyed in that study were a randomly selected subset of Adirondack lakes. In the present study, I examined data from all lakes in that subset having a pH below 5.0, including both clearwater, anthropogenically-acidified, and humic, naturally acidic lakes. The lakes were sampled once during summer 1984 for chemistry, plankton and fish. Chemical and phytoplankton samples were taken at mid-epilimnion, and zooplankton were collected with vertical tows of a plankton net (76 μm mesh for rotifers, 158 μm mesh for crustaceans). Water chemistries were done using standard U.S.E.P.A. methods, phytoplankton were enumerated using the Utermöhl technique, and zooplankton were enumerated in counting chambers. Fish were sampled with overnight gill nets and minnow traps.

Food Web Construction

A cumulative list of the species collected in the 28 acidic lakes was produced. As previously explained (Havens 1993a), the term species refers to biological taxa, rather than trophic species. In some cases, taxa were reported as unidentified but separate species within a genus, e.g., "*Mallamonas* species A." Pre-adult categories were included for species that undergo ontogenic changes in diet or susceptibility to predators. Those categories were nauplii, fish fry, and fish eggs. I also included the category "detritus," as a food source for benthivorous fish and cladocerans. Species' diet information was based on personal observations (Havens and DeCosta 1985), and on the published results of other researchers, in particular, Scott and Crossman (1973), Bogdan and Gilbert (1984), Sprules and Bowerman (1988), and Martinez (1991). That information was used to construct a cumulative matrix of predator–prey interactions among the taxa. The matrix consisted of rows representing prey and columns representing predators. Each a_{ij} element in the matrix was assigned a value of unity if the ith prey was preyed upon by the jth predator, a zero if not. Food web matrices were then constructed for each lake, by extracting from the cumulative matrix those rows and columns corresponding to the species present in each lake. Four food web matrices were edited by deleting species (a total of five) which lacked trophic links to the webs.

The cumulative matrix approach assumes constant predator–prey interactions. That is, if species A eats B in one lake, it eats B in all other lakes where both are present, regardless of the presence of alternative prey. This assumption may not be valid. For example, predators might change their diets in species-poor communities, and predators in species-rich communities might not eat all potential prey. Hence, the linkage complexity of species-rich webs might be overestimated by the approach taken herein. The fact that particle feeders (zooplankton and zooplanktivorous fish) are the dominant predators in these lake webs likely minimizes the bias. Unlike piscivorous fish, which search out and capture individual prey, particle feeders consume food items falling within a particular size class, and the number of food types exploited is potentially unlimited (Havens 1993b).

The optimal approach to constructing food webs would be to directly establish all food web links. However, that would require gut content analyses or controlled feeding experiments with all pelagic predators in each lake. Such an approach would limit the study to a small number of lakes.

TABLE 1. Correlations between pelagic food web attributes and selected abiotic variables in 28 acidic Adirondack lakes. An asterisk indicates a correlation coefficient that is significant at $p = 0.05$. S = number of species, L = number of links, L/S = linkage density, H^+ = hydrogen ion ($\mu eq \cdot L^{-1}$), mAl = monomeric Aluminum ($\mu eq \cdot L^{-1}$), DOC = dissolved inorganic carbon ($\mu eq \cdot L^{-1}$), TP = total phosphorus ($\mu eq \cdot L^{-1}$), Area = lake area (ha).

	S	L	L/S
H^+	−0.22	−0.28	−0.28
mAl	−0.25	−0.30	−0.36
DOC	+0.37*	+0.38*	+0.40*
TP	+0.30	+0.40*	+0.41*
Area	−0.10	−0.17	−0.22

Web Analyses

For each food web, three structural attributes were determined. They indicate the number of different kinds of taxa, and the complexity of the interactions among them: (1) number of species (S) = total number of biological taxa, (2) number of links (L) = total number of predator-prey interactions, and (3) linkage density (L/S) = total number of links divided by the total number of species.

Relationships between the three food web attributes and several abiotic variables (DOC, monomeric Al, H^+, total P, and lake area) were tested statistically using correlation and stepwise multiple regression procedures. The relationships between DOC concentration and the food web attributes were non-linear, and the DOC data were log-transformed prior to the analyses.

Results and Discussion

In a previous investigation (Havens 1993a), I found that with declining pH, pelagic food webs of Adirondack Mountain lakes become greatly simplified. Locke (1992) and I showed that in acidic lakes there is also great variation in food web complexity. This was attributed (Havens 1993a) to differences in toxic Al concentrations among clearwater and humic lakes. The present results confirm that notion, because the high DOC, humic lakes (wherein Al ions are presumably bound to organics and rendered non-toxic) contained food webs with substantially greater size and complexity than low DOC, clearwater lakes.

In the acid lake food webs, the number of species, links and the linkage densities were positively correlated with the log-transformed DOC concentrations (Table 1). Links and linkage densities were also correlated with total phosphorus concentrations. No other relationships were significant. Stepwise multiple regression yielded models that incorporated log-transformed DOC concentration as the primary variable and H^+ concentration as a second predictor (Table 2). The models explained over 35% of the inter-web variation in the food web attributes; most of the variation was explained by DOC. Species, links and linkage density increase rapidly as DOC increase from < 2 to roughly 4 $mg \cdot L^{-1}$ (Fig. 1). At higher DOC levels, the food web attributes reach plateaus of roughly 35 species, 120 links, and 4 links per species.

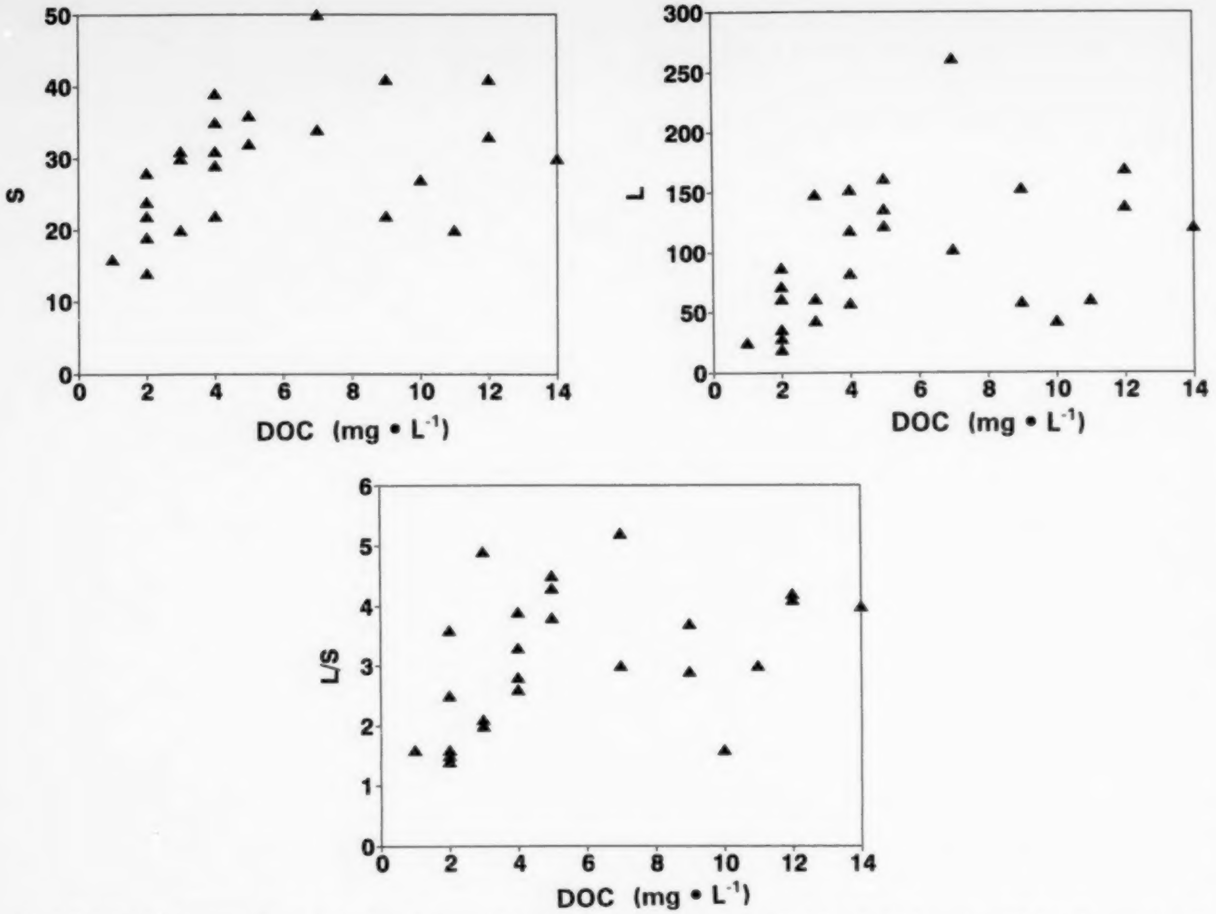

FIG. 1. Relationships between three food web attributes and lakewater DOC concentration for 28 Adirondack Mountain acid lakes. S = number of species, L = links, L/S = links per species.

TABLE 2. Results of stepwise multiple regression analyses of pelagic food web attributes in relation to abiotic variables in 28 acidic Adirondack lakes. The independent variables tested were DOC, H$^+$, mAl, TP and area. The R^2 for entry into the model was 0.05, and the DOC data were log-transformed prior to the analysis. Symbols are as defined in Table 1.

Parameter	Variable	F	$p > F$	Model R^2
S	DOC	13.26	0.001	
	H$^+$	4.33	0.046	0.37
L	DOC	14.65	0.001	
	H$^+$	7.22	0.013	0.42
L/S	DOC	16.25	0.001	
	H$^+$	7.66	0.011	0.44

Overall, the acidic lakes having high levels of DOC contained more species, and had more complex food webs. Similar trends have been reported for phytoplankton, rotifers and crustacean zooplankton. Siegfried et al. (1989a) surveyed the phytoplankton of the Adirondack lakes studied herein (plus 22 additional lakes with pH > 5.0), and found distinct clusters of acid lakes. One cluster contained lakes that were high in both DOC and total Al. Those lakes contained, on average, 20 phytoplankton species; more than any other acid lake cluster. The authors suggested that DOC ameliorated the toxic effects of Al on the phytoplankton. A second lake cluster was also characterized by high Al levels, but the lakes were "clearwater" (low DOC). The phytoplankton contained, on average, only 13 species. The authors noted that those lakes were fishless, and attributed both the absence of fish and the lower phytoplankton diversity to aluminum toxicity. Siegfried et al. (1989b) surveyed the rotifer populations and obtained similar results. The largest cluster contained mostly alkaline lakes. However, seven highly colored acid lakes with diverse rotifer assemblages (9–14 species) were included in the cluster. Two other clusters contained clearwater acid lakes, with high Al levels, and sparse rotifer assemblages (≤5 species). Sutherland et al. (1989) surveyed the crustacean zooplankton. They reported that diversity indices (H) were low in most acid waters; the exceptions were acid lakes with high DOC concentrations. My results integrate this information from the various plankton trophic levels, and also include fish. They show that in acidic Adirondack lakes, both the overall species richness and the complexity of the food web are strongly regulated by the concentration of DOC in the water. The magnitude of the DOC effect is remarkable; this single chemical variable explains nearly 40% of the variation in pelagic food web structure. And, the result is consistent with a hypothesis (Locke 1992) that biotic factors like competition and predation play little role in determining community structure in acid stressed lakes, where instead, chemical toxicants are the major regulators.

The form of Al measured in the Adirondack survey was "total monomeric," which included both toxic inorganic and less toxic organic species. Hence, it was impossible to establish a direct relationship between toxic Al levels, DOC, and food web structure. Nevertheless, the non-linear relationships between the food web attributes and DOC support the view that the DOC effect is amelioration of Al toxicity. The trends in species, links, and linkage density suggest a "saturation" at roughly 4 mg $DOC \cdot L^{-1}$. At that level of DOC, most of the Al present in the acid lakes (roughly 50–600 $\mu g\ Al \cdot L^{-1}$) may be complexed. Because the chemical form of DOC is unknown, it is impossible to test this view by calculation. However, Driscoll et al. (1980) have shown that at 2 mg $DOC \cdot L^{-1}$, a substantial fraction of the total Al is complexed. Baker et al. (1990) noted that "measurements of DOC may be useful in assessing the potential for Al toxicity, particularly in the absence of measurements of labile (inorganic) monomeric Al." It has been clearly documented that once complexed, Al is markedly less toxic to biota. Baker and Schofield (1980) found that 500 $\mu g\ Al \cdot L^{-1}$ caused 80% mortality of brook trout fry at pH 5.2, but when 30 mg $citrate \cdot L^{-1}$ was added with the Al, mortality was reduced to 5%. Neville (1985) found that at pH 5.0 and 10 mg $DOC \cdot L^{-1}$, 300 $\mu g\ Al \cdot L^{-1}$ was required to produce a toxic response in rainbow trout equivalent to that produced by 75 $\mu g\ Al \cdot L^{-1}$ and no DOC. Likewise, McCahon and Pascoe (1989) recorded high mortalities of macroinvertebrates and fish in stream segments treated with acid (pH 4.9) and Al (270 $\mu g \cdot L^{-1}$), but low mortalities in segments where citrate (0.7 $mg \cdot L^{-1}$) was also added along with the acid and Al. Freda et al. (1990) recorded an LC50 of 471 $\mu g\ Al \cdot L^{-1}$ for *Bufo americanus* tadpoles. When 12 mg $Al \cdot L^{-1}$ was included in the soft water medium, the LC50 was increased to over 1000 $\mu g\ Al \cdot L^{-1}$. Although these results may not be directly comparable to the present study (due to the use of artificial DOC in bioassays), they do show that DOC can ameliorate Al toxicity at the Al levels observed in the acidic Adirondack lakes. My results indicate that the DOC effect is important at the level of the entire food web. There is clearly a need for controlled whole-community experiments, to test specific hypotheses about acid, Al and DOC effects on pelagic food web structure and function.

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Behavioral Responses of *Drunella coloradensis* (Ephemeroptera) Nymphs to Short-Term pH Reductions

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Pennuto, C.M., and F. deNoyelles, Jr. 1993. Behavioral responses of *Drunella coloradensis* (Ephemeroptera) nymphs to short-term pH reductions. Can. J. Fish. Aquat. Sci. 50: 2692–2697.

Behavioral responses of *Drunella coloradensis* nymphs were examined in outdoor experimental stream channels after pH reductions of 1 and 2 pH units below ambient. The severity of pH decline below the ambient of 7.8 influenced the behavior patterns displayed by nymphs. At pH 7.01 (an intermediate pH decline) nymphs sat less frequently and burrowed more than controls. Burrowing behavior frequency returned to control levels and drifting and crawling behaviors increased relative to controls at pH 6.02. Ventilatory behaviors increased with pH decline, but were independent of the severity of acidity increases. These results suggest that individual behaviors may offer a more sensitive indicator of sub-acute stress in aquatic insect communities than population or community monitoring. Behaviors leading to increased activity levels in stream insects may have community-level effects via changes in predator–prey encounter rates or increased susceptibility to passive drift. These potential changes are discussed in reference to monitoring for acidification effects.

On a étudié les comportements de nymphes de *Drunella coloradensis* dans des tronçons de cours d'eau expérimentaux après une réduction de 1 et 2 unités de pH par rapport au pH ambiant. La gravité de la baisse de pH par rapport au pH ambiant de 7,8, a influencé les comportements manifestés par les nymphes. À pH 7,01 (soit une baisse intermédiaire du pH), les nymphes se posaient moins fréquemment et s'enfouissaient davantage que les témoins. La fréquence du comportement d'enfouissement retrouvait celle des groupes-témoins et les comportements de dérive et de rampement se faisaient plus fréquents par rapport aux témoins, à pH 6,02. Les comportements ventilatoires se sont accrus à mesure que le pH baissait, mais ils étaient indépendants de la gravité de l'acidification. Ces résultats donnent à penser que les comportements individuels peuvent constituer un indicateur plus sensible du stress subaigü dans des communautés d'insectes aquatiques que ne l'est la surveillance des populations ou des communautés. Des comportements qui conduisent à une activité accrue chez les insectes lotiques peuvent avoir des effets au niveau de la communauté dans la mesure où sont modifiés les taux de rencontre entre prédateurs et proies ou encore est accrue la susceptibilité à une dérive passive. Il est question de ces changements en termes de surveillance des effets de l'acidification.

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Aquatic ecologists have demonstrated that reduced pH may have negative effects on individuals, populations, and communities of aquatic insects. To assess the potential impacts of acidification in lotic systems, researchers have monitored community level effects on benthic density, diversity, or functional group abundance (Friberg et al. 1980; Hall et al. 1980; Hildrew et al. 1984; Allard and Moreau 1987; Hopkins et al. 1989; Hall 1990). Studies concerning individual responses to acidification have focused on physiology, body mass changes, and survival or emergence (Bell 1971; Fiance 1978; Havas and Hutchinson 1983; Allard and Moreau 1986; Correa et al. 1986; Herrmann and Andersson 1986; Rowe et al. 1988, 1989; Mackie 1989; Chmielewski and Hall 1992). Drift rates are probably the most widely examined response to acidification (Hall et al. 1980; Pratt and Hall 1981; Ormerod et al. 1987; Hopkins et al. 1989; Bernard et al. 1990): generally drift increases in most taxa. Grazing mayflies (e.g., baetids and siphlonurids), and small chironomid midges (Orthcladiinae) appear to be the most sensitive insects to reduced pH. Although increased drift rates have been documented with experimental acid additions, it is unknown whether this drift response is active, passive, or a combination of these. Other factors (both genetic and environmental) might also act to increase or decrease the likelihood of drift and no research demonstrates whether other behaviors in insects are altered under acid stress (but McCahon et al. (1989) have addressed changes in feeding rates of amphipods and snails in relation to increased acidity). The importance of insect behaviors in response to acidification and the implications of behavioral alterations in determining the outcome of higher level interactions has not yet been addressed.

Aquatic insects might use behaviors such as drifting or burrowing to evade exposure to short-term increases in acidity. One might expect some behaviors to predominate depending on the severity of the disturbance (Resh et al. 1988) and the energetic trade-offs between behavior and physiology (Dill 1987). In particular, regulatory behaviors (e.g., Wiley and Kohler 1984) often involve conflicts with other behaviors and are likely to be the most sensitive indicators of stress. For example, under low oxygen conditions metabolic carbon dioxide must be counteracted before tissue acidosis occurs (Nation 1985). Mayflies might increase gill ventilation rates to overcome a minimal oxygen debt or drift if the oxygen stress is severe (Eriksen 1963; Wiley and Kohler 1980). Drifting, however, may pose added predation risks from visually feeding fish (Waters

1972; Allan 1978, 1984; Stewart and Szczytko 1983; Skinner 1985). Thus, a conflict would arise if predation risk was high and oxygen stress was greater than could be overcome by increases in ventilation rate.

As with a low oxygen stress, increased acidity may lead to a physiological disruption that requires rectification. In mayflies, metabolic ion concentrations are disrupted with increasing acidity (Rowe et al. 1988, 1989; but see Berrill et al. 1991), and depending on the costs, certain behavioral responses might be expected as counter measures. For example, behaviors which ensure adequate evasion of the stress, yet do not increase predation risks, might be favored. If behavioral responses to stress maximize regulatory efficiency based on energetic trade-offs, drift (a physiologically inexpensive, direct escape) should increase under stressful conditions (i.e., reduced pH) if there are no other associated costs. If there are costs to drifting, behaviors other than drifting may be even more sensitive indicators of acid stress in aquatic insects. At low-level pH stress, trade-offs may exist between risks associated with physiological intolerance and predation such that drifting is avoided until physiologically necessary.

Here we report the results of a replicated experimental stream study in which we reduced ambient pH and observed *Drunella coloradensis* nymphs for behavioral changes. We predicted that: (1) nymphal behavior would be influenced by low-level, short-term pH reductions and (2) drifting (a potentially expensive behavior in terms of predation, but energetically inexpensive) would occur more often at the lowest pH levels examined compared to lesser pH declines. Our results suggest that nymphal behavior is influenced by acidity increases, but to different degrees depending on the severity of pH reductions. We discuss these results in reference to monitoring for acidification effects and the implications of behavioral alterations in response to stress.

Study Site and Methods

Site Description

Two montane streams in the Rocky Mountains of southeast Wyoming were used; Little Brooklyn Lake inlet (LB) and Mullen Creek (MC). LB is a second order stream at 3158 m elevation in a remote location of the Medicine Bow National Forest in southeast Wyoming (41°22′W, 106°15′N). MC is a first order stream at 2572 m elevation located adjacent to the United States Forest Service (USFS) research station 1 km west of Centennial, WY (41°18′W, 106°09′N). Substrate, flow and water chemistry, especially pH and alkalinity, are similar between the LB and MC sites, which are approximately 10 km apart (Table 1). Water chemistry in LB is monitored routinely by USFS personnel as part of their Glacier Lakes Ecosystem Experiments Site (GLEES).

Both sites occur within forest habitat dominated by lodgepole pine (*Pinus contorta*), Ponderosa pine (*Pinus ponderosa*), and scattered stands of quaking aspen (*Populus tremuloides*). Both sites are fed primarily by snowmelt, but LB has a lake approximately 2 km upstream while MC has no upstream lake. MC and LB both contain brook trout populations (*Salmo funeralis*).

Study Organism

The mayfly *Drunella coloradensis* (Dodds) (Ephemerellidae) is widely distributed and generally occurs at elevations greater than 1700 m (Jensen 1966; Ward 1985). Like most emphemerellids, *D. coloradensis* is a poor swimmer, generally slow-moving, and exhibits a clinging/sprawling life style (Edmunds 1984). Primary food items include diatoms at early instars and animal matter during later instars. In Oregon this species exhibits a univoltine life history and shows very synchronous nymphal growth (Hawkins 1990). Adults emerge in late summer and early autumn (Jensen 1966; personal observation). This species occurs in both LB and MC, but in low numbers in MC.

TABLE 1. Water chemistry for the Mullen Creek (MC) study site and Little Brooklyn Lake inlet (LB) nymph collection site on 8 August 1991. All measurements are in μeq/L unless stated otherwise.

Variable	Site	
	MC	LB
pH	7.80	7.49
Temperature (°C)	11.0	12.0
Conductivity (μS/cm^2)	111.87	69.91
Alkalinity	1127.58	1156.38
Ca^{2+}	871.66	807.63
Mg^{2+}	203.57	251.64
Na^+	201.92	18.40
K^+	22.20	6.30
NH_4^+	1.45	3.06
Cl^-	10.92	2.02
NO_3^-	0.06	1.86
SO_4^{2-}	92.03	18.61

Experimental Procedures

Drunella coloradensis nymphs were collected from LB on 31 July and 6 August 1991. Nymphs were picked from stones using a fine brush or forceps, placed in a cooler with LB stream water, and transported to the study reach on MC, nymphs were placed in in-stream enclosures for at least 48 h prior to use in experiments. In-stream enclosures were weighted sieving buckets and contained MC substrate with attached periphyton. Only insects that responded to stimulation with a brush were used for experiments. Nymphs were identified using keys of Edmunds (1984) and Ward (1985).

Experimental stream chambers were constructed of clear Plexiglas. The chambers measured 57.0 × 33.5 × 20 cm with a 10-cm collecting basin at each end (Fig. 1). Five panels divided each chamber into six, 5 × 34.5-cm stream channels. A 1-mm mesh plastic screen served as a barrier on the upper and lower channel ends. In each experimental chamber, stream water was recirculated with a magnetic, centrifugal pump (Little Giant, Model 3-MD-SC) and transferred through a series of Teflon tubes such that channel velocity was within 2 cm/s of the velocity at the LB collection site.

Three experimental chambers were imbedded 2–5 cm within the substrate of MC to maintain slope and contact with surrounding water. Thus the stream served as a bath to maintain the water temperature within the chambers. Each chamber represents one of three pH treatments: an ambient control (pH approx. 8.0), 1 pH unit below ambient, and 2 pH units below ambient. Treatments are referred to as C, -1, and -2, respectively. The six channels in each chamber received 2–3 cm of stream substrate (a sand and gravel mixture) that had been sifted to remove any organisms. Chambers were filled with 12 L of MC

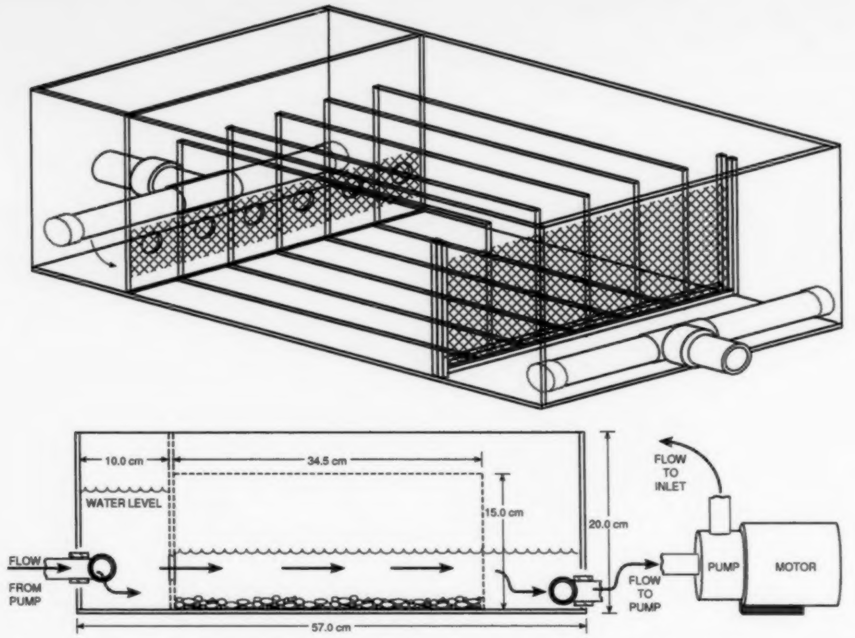

FIG. 1. Diagram of experimental stream chambers used for examining behavioral responses to acidification. Chambers are self-contained, recirculating units powered by magnetic, centrifugal pumps. Chamber measurements (length, width, and height) are 57.0 × 33.5 × 20.0 cm. Each chamber is subdivided into 6 channels measuring 34.5 × 5.0 × 15.0 cm (l,w,h, respectively). There is a 10-cm wide collecting basin at each end of a chamber. The units are placed in-stream for temperature regulation.

stream water and the water was circulated in the chambers for 24 h prior to insect and acid additions.

Each channel received one *D. coloradensis* nymph (six nymphs per treatment per day). Nymphs were acclimated to channels for 24 h prior to acid additions. At 09:00 on 2 and 8 August enough 0.01 *M* H_2SO_4 was added to reduce pH levels in two chambers -1 and -2 pH units below ambient (control pH = 7.79, -1 pH = 7.01, -2 pH = 6.02). Continuous recording pH meters (Photovolt Aquacord Model 130) monitored pH levels and manual acid additions were used to maintain pH levels. Water samples for chemical analyses were collected from each chamber at the start and the conclusion of each experiment. Samples were analyzed for major anions and cations by the USFS Water Chemistry Lab in Ft. Collins, CO.

Scan sampling of behaviors was performed at 5-min intervals during the first 2 h following acid additions. Scan sampling requires an observation of each individual after a fixed amount of time (Martin and Bateson 1986). Because each channel contains only one nymph, an observer could scan each of the 18 channels (six per treatment) for a nymph, record its behavior, and continue through the remaining channels. A "scan" was completed within 5 min and the procedure was repeated 25 times. The behaviors of sitting (*s*), ventilating (*v*), burrowing (*b*), drifting (*d*), crawling (*c*), and ventilating while crawling (*vc*) were recorded. "Sitting" represented a nymph sitting on an exposed substrate surface without movement of the gills or body. "Ventilating" represented a nymph with visible gill movement sitting on an exposed substrate surface. "Burrowing" was recorded for a nymph not visible drifting or visible on the surface of any substrate. "Drifting" represented a nymph in the water column. "Crawling" was recorded for a nymph moving across the substrate without visible gill movement. "Ventilating while crawling" was recorded for nymphs moving across the substrate while having visible gill fluttering.

The total frequency of behavioral responses was tested for independence of pH treatment using a Kruskal–Wallis one-way ANOVA. A posteriori multiple comparisons within behaviors were based on a modified Mann–Whitney *U*-test (Sokal and Rohlf 1981). The frequency of behaviors was not significantly different between trial days for 16 of 18 comparisons. Because there were only two significant differences, we pooled all behaviors from the two trial days. We feel pooling caused statistical analyses to be more conservative (i.e., less likely to show a significant treatment effect if one did not occur) because of the additional variance within comparison treatments and was appropriate in this study.

Results

Water chemistry changed as expected after acid additions. Alkalinity and pH levels declined while conductivity and SO_4^{2-} levels increased (Table 2). Changes in the major cations (Ca^{2+}, Mg^{2+}, Na^+, and K^+) were somewhat erratic, but all generally increased with acid additions; anions also tended to increase.

Acid treatment influenced the frequency of behaviors displayed by *D. coloradensis* nymphs, but did not prevent or change any behavior (i.e., nymphs exhibited all behaviors under all treatments, but at varying frequencies). Kruskal–Wallis ANOVA results suggest that all behavioral frequencies were altered by at least one treatment (Table 3).

TABLE 2. Water chemistry in experimental chambers before acid additions and after 2-h experiments. The pH was reduced using 0.01 M H_2SO_4. Ions are in μeq/L unless stated otherwise.

Variable	Before			After		
	Control	-1	-2	Control	-1	-2
pH	7.79	7.81	7.81	7.92	7.01	6.02
Temperature (°C)	10.2	10.5	10.1	11.0	11.1	10.9
Conduct. ($\mu S/cm^2$)	112.2	110.2	113.0	111.7	127.6	143.3
Alkalinity	1194.8	1148.3	1126.1	1184.2	446.7	56.2
Ca^{2+}	953.3	825.7	815.9	828.1	836.1	873.0
Mg^{2+}	210.4	202.8	197.5	202.4	195.9	212.5
Na^+	200.6	201.4	203.7	201.1	203.8	208.9
K^+	20.9	20.8	23.4	21.8	22.9	27.3
NH_4^+	1.3	1.7	1.4	1.6	1.3	1.2
Cl^-	10.2	10.2	12.3	10.9	12.6	15.9
NO_3^-	0.0	0.0	0.0	0.0	0.0	0.0
SO_4^{2-}	94.8	110.8	144.9	97.0	768.6	1105.7

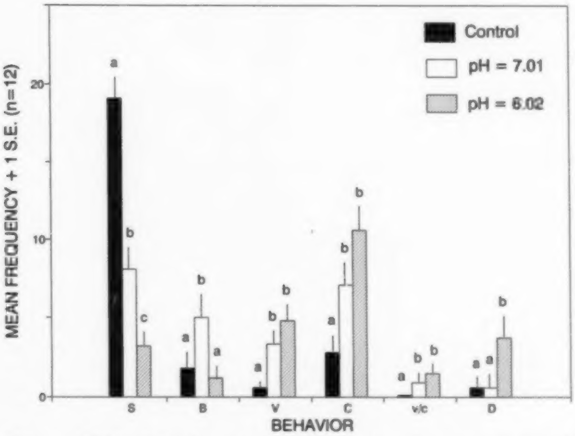

FIG. 2. Within behavior comparisons for larval responses to acid additions. Multiple comparisons within behaviors were tested using a modified Mann–Whitney U-test. Three comparisons were made for each behavior (C vs. -1, C vs. -2, and -1 vs. -2). Same letters within a behavior indicate the treatments were not different at $P < 0.05$. See text for P values of significant differences. On behavior axis, S = sitting; B = burrowing; V = ventilating; C = crawling; v/c = ventilating while crawling; and D = drifting.

TABLE 3. Mean frequency (±1 SE) of observed behaviors for *D. coloradensis* nymphs after acid addition experiments (n = 12 nymphs/treatment). Nymphs were observed at 5-min intervals during the 2-h experiments. Control: pH = 7.81, -1: pH = 7.01, -2: pH = 6.02. See text for explanation of behaviors. H = Kruskal–Wallis statistic examining treatment effects on behaviors. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Behavior	Treatment			
	Control	-1	-2	H
Sit	19.1 (1.25)	8.1 (1.20)	3.2 (0.76)	24.777***
Burrow	1.8 (0.80)	5.0 (1.45)	1.2 (0.64)	8.238*
Drift	0.6 (0.22)	0.6 (0.48)	3.7 (1.06)	10.308**
Ventilate	0.6 (0.27)	3.3 (0.63)	4.8 (0.80)	18.158***
vc	0.1 (0.08)	0.9 (0.32)	1.5 (0.34)	13.399**
Crawl	2.8 (0.81)	7.1 (1.26)	10.6 (0.82)	15.689***

The frequency of sitting declined with increasing acidity (Fig. 2). The control nymphs sat more frequently than any other behavior. Control nymphs sat approximately 75% of observations and this was more than both -1 and -2 nymphs (Mann–Whitney U-test, $P < 0.001$, and $P < 0.001$ respectively). The -1 treatment nymphs also sat more than -2 nymphs ($P < 0.05$; Fig. 2) and sitting was the most frequent behavior recorded for the -1 nymphs (about 32% of observations).

Burrowing behavior increased at intermediate acidity levels and then declined back to control frequencies at higher acid treatment levels (Fig. 2). Burrowing of -1 nymphs was greater than both control and -2 nymphs (Mann–Whitney U-test, $P < 0.01$ and $P < 0.05$), but was never the most frequent behavior recorded. Burrowing behavior was recorded for 20% of observations at intermediate pH levels, but less than 10% of control and -2 observations.

Crawling (c), ventilating (v), and ventilating while crawling (vc) showed a similar trend with pH. The -1 and -2 treatment nymphs were not significantly different from each other in these three behaviors, but were different from the controls for these behaviors (Cc vs. -1c, Cc vs. -2c, Cv vs. -1v, Cv vs. -2v, Cvc vs. -1vc, Cvc vs. -2vc; U = 112.0*, 137.5***, 130.5***, 134.5***, 109.0*, 128.0**, respectively). Crawling was the most frequent behavior recorded for the -2 nymphs, occurring 42.4% of observations. Behaviors of v and vc were never the most frequently recorded behaviors, but were more frequent for -2 nymphs relative to other treatments (19.2 and 6% respectively).

Drifting behavior occurred more frequently in -2 nymphs than control and -1 treatments (Mann–Whitney U-test, $P < 0.05$). Drift rates showed an approximately 6-fold increase in the -2 treatment compared to both -1 and control treatments. Drifting was the third most frequent behavior observed in -2 nymphs, but the least frequent behavior in control nymphs (Fig. 2). Drifting behavior showed no significant increase at intermediate pH levels. Drifting was not correlated with crawling for the control, -1 or -2 treatments (Spearman's rho, all $P > 0.05$).

Discussion

The frequencies of behaviors displayed by *D. coloradensis* nymphs were influenced by acidity in our experiment and depended on the severity of pH reduction. In general, as acidity levels increased, activity levels also increased (e.g., crawling was more frequent in the treatment chambers than in the controls). Early toxicity research suggested that increased activity, including crawling, loss of equilibrium, and drifting, might be a generalized, first response mechanism to physiological stress (Jensen and Gaufin 1964a, 1964b; Muirhead-Thomson 1978). It is also interesting that endoparasitic infections also cause increased activity levels in some mayfly species (Benton and Pritchard 1990). Increased movement patterns might alter interactions between stream insects by increasing encounter rates with predators (Soluk and Collins 1988) or by increasing the probability of abrasion by water currents (Poff et al. 1991).

Peckarsky (1980, 1982, 1991) and Peckarsky and Dodson (1980) showed that behavioral interactions (especially predator/prey) are important in structuring lotic insect communities under natural conditions. Behavioral responses to environmental stress (especially involving physiological adjustments) may have grave implications to these interactions if the species within a community exhibit some tolerance differences to the stress. For example, Warner et al. (1993) showed that pH tolerance differences in anuran tadpoles, when combined with population density differences, may alter the outcome of inter- and intraspecific competition even at pH levels not resulting in death. Likewise, Clements et al. (1989) showed that sublethal copper exposure increased the ability of predatory stoneflies to capture netspinning caddis flies because the stoneflies were more tolerant of copper than were the caddis larvae. In our experiment, the increased crawling behavior displayed by mayfly nymphs might result in increased encounter rates with predators, reduced ability to detect predators, or an increased likelihood of passive drift due to abrasion by currents. If mayfly nymphs are less tolerant of pH stress than are their stonefly predators, and if a general response to stress is evasion, stonefly predators might experience higher prey capture rates either because of increased prey encounters or because prey are less capable of detecting predators.

As with crawling, increases in drifting rates may influence encounter frequency between species. Drifting frequency increased at the lowest pH level examined in our experiment, but not dramatically. Drift responses might have been dampened because the pH reductions in this experiment were not very severe, *D. coloradensis* might be very tolerant of acidity, or drifting may be phylogenetically or morphologically constrained in this species. Also, scan sampling probably gave conservative measurements of drifting behavior because drifting bouts of shorter duration than scan intervals would be missed. Depending on natural drift distances, short channel length might have led to an over- or under-estimate of drifting frequency because drifters encountered the retaining net. If drift was an active evasion tactic, nymphs would be expected to remain on the downstream end of the channels attempting to reenter the water column (i.e., drifting to avoid stress). Nymphs did not remain on the screen, but generally crawled back to the substrate.

Another active behavior, burrowing, may negate the consequences of increased crawling and drifting due to acid stress. At intermediate pH, burrowing may be a pH-stress avoidance behavior that is risk-free in terms of predation if predators are less able to burrow than are prey. Also, ion-exchange dynamics within the substrate (or any material where an insect can hide) may be different enough that pH levels are more stable (Likens 1989), offering a potential refuge from pH stress. Weatherley et al. (1989) showed that pH levels were positively related to increasing depth within the substrate and that pH was up to 2 units higher in the substrate than in the overlying water. This relationship must ultimately depend on the nature of the substrate. The value of burrowing as an evasion tactic will depend on this and a combination of biotic and abiotic factors and their relative costs. Body morphology, nature of the substrate, cost of drifting, and predation risks within the substrate are examples of potential factors determining the likelihood of burrowing as an escape strategy. In general, small-bodied nymphs should burrow better than large-bodied nymphs because of access to interstitial spaces. Examination of size distributions of drift and benthos between pre- and post-acid additions under experimental conditions might address this possibility. Likewise, nymphs with fusiform bodies might be expected to burrow better than dorso-ventrally flattened nymphs (i.e., baetids vs. heptageniids). Swimming ability and propensity to drift would require attention when interpreting such data.

The use of inadequate endpoints might lessen our ability to interpret results when monitoring for acidification effects. This study suggests that current sampling protocols may, in fact, under represent potential impacts and that behaviors may provide an earlier indication of acid stress in insect communities. Routine monitoring of insect communities for acidification effects generally include drift, benthic, and emergence estimates (e.g., Hall et al. 1980). Our results suggest there are lower thresholds of stress (i.e., intermediate pH declines influencing behavior) that would not be detected with drift estimates. Benthic estimates using Surber-type samplers give no indication whether insects are hiding in the substratum or normally reside there. Possibly, examinations of lateral fringe or within-leafpack communities would be better methods to estimate refuge seeking behavior. Behaviors might also offer useful tools in toxicological research and field testing for potential hazards by yielding more realistic estimates of first-effects concentrations of stressors. Here, using a simple, replicated observation method we have demonstrated that behavioral responses of a common, lotic mayfly are altered dramatically by a low-level, pH disturbance. Regardless of how monitoring protocols are devised in the future, it is clear that behaviors will have to be incorporated into sampling designs.

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Toxic Metals in Pilot Whales (*Globicephala melaena*) from Strandings in 1986 and 1990 on Cape Cod, Massachusetts

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Concentrations of As, Cd, Cu, Hg, Pb, and Se were measured in 17 adult and 8 fetal pilot whales (*Globicephala melaena*). Total Hg and Se both occurred in very high concentrations in liver and kidney and in liver were significantly correlated with animal length (and each other) which indicates bioaccumulation over time. Methyl mercury, as a percentage of total Hg, varied inversely with total Hg indicating demethylation was occurring; a one-to-one molar association of Hg and Se was found which is believed to provide protection against Hg toxicity. Arsenic concentrations were relatively low, but Cd concentrations were consistently very high in adult kidney. Lead in adult liver was also correlated with animal length indicating long term bioaccumulation. All non-essential elements were found in critical fetal tissues which indicates maternal transfer occurred. Cadmium in fetal kidney was over 30 times higher than either brain or liver, indicating early differential accumulation and supporting maternal transfer. Matched pairs of mother and fetus liver indicated no correlation of elements, which may due to the temporal nature of bioaccumulation and the immobility of some elements. These results were compared to other mammalian studies to evaluate the toxicological implications of observed tissue concentrations.

On a mesuré les concentrations d'As, de Cd, de Cu, de Hg, de Pb et de Se dans les tissus de 17 globicéphales noirs (*Globicephala melaena*) adultes et 8 foetus. Le Hg total et le Se étaient tous deux trouvés en très fortes concentrations dans le foie et les reins; il y avait une corrélation significative entre les concentrations dans le foie et la longueur de l'animal (et entre elles); cela indique une bioaccumulation. Le mercure-méthyl, exprimé en pourcentage du Hg total, est en relation inverse avec la concentration de Hg total; cela est indicatif d'une déméthylation. On a trouvé une association molaire dans une proportion de un à un entre le Hg et le Se; on pense que cela peut conférer une protection contre la toxicité du Hg. La concentration en arsenic est assez faible, mais la concentration en Cd était régulièrement très élevée dans les reins des sujets adultes. Le plomb trouvé dans le foie des adultes était aussi en corrélation avec la longueur de l'animal; cela est indicatif d'une bioaccumulation à long terme. Tous les éléments non essentiels ont été trouvés dans les tissus foetaux d'importance critique; cela pointe vers un transfert mère-foetus. Dans les reins des foetus, le cadmium était 30 fois plus concentré qu'il ne l'était dans le cerveau ou le foie; cela indique qu'il se fait tôt une accumulation différentielle et un transfert de la mère à l'enfant qui alimente ce phénomène. Des paires appariées de foies de la mère et du foetus n'ont pas montré de corrélation avec les éléments; cela peut s'expliquer par le fait que la bioaccumulation se manifeste avec le temps ainsi que par l'immobilité de certains éléments. Ces résultats ont été comparés à ceux d'autres études sur des mammifères afin d'évaluer la signification sur le plan toxicologique des concentrations observées dans les tissus.

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Mass strandings of Cetaceans are relatively common around the world but most of them remain basically unexplained. There are several theories and probably many reasons why these animals strand en masse (Brown 1975; Stoneburner 1978; Geraci 1989) but virtually none have been conclusively tested. At least one paper has pointed out that mass strandings have been observed for the last 200 years (Nores and Perez 1982). Mass strandings of pilot whales (*Globicephala* sp.; Delphinidae) are fairly common. Strandings of individuals of *Globicephala* sp. have been recorded in Newfoundland (McLeod 1986; Muir et al. 1988), Chile (Venegas and Sielfeld 1980), Spain (Nores and Perez 1982), England (Fairly 1981), southern California (USA) (Hall et al. 1971), Cape Cod (USA) (Fine 1983), and Georgia (USA) (Stoneburner 1978).

Toxic chemicals are one class of agents suspected of being involved in affecting the health of marine mammals and leading to instances of mass stranding. Researchers have analyzed marine mammal tissue for metals and metalloids routinely for years attempting to link effects or exposure with high body burden. High levels of mercury, and other elements, have been noted in beached and stranded animals, as well as those caught in nets or harvested (which were presumably healthy at the time of capture). In general, several elements can be high in both groups, which makes interpretation of any toxic effects difficult.

Another factor limiting the ability to assess toxicity is the interaction between chemical contaminants. One of the better known correlations of elements in marine mammals is that of Hg and Se in liver and kidney that, in many cases, occurs in a one-to-one molar ratio (Kari and Kauranen 1978; Koeman et al. 1973, 1975; Itano et al. 1984; Pelletier 1985, for a review see Cuvin-Aralar and Furness 1991). Mercury and selenium can accumulate to very high levels in marine mammals, often exceeding levels that would be considered lethal. Several laboratory studies with small mammals have determined that mercury is much less toxic when accompanied by an equivalent dose of selenium (Ganther et al. 1972; Ohi et al. 1980). One study has shown that inorganic selenium, in the presence of glutathione, can demethylate methyl mercury (MeHg) and form an insoluble complex (Iwata et al. 1982). In the marine environment, this relationship has been observed only in marine mammals and true antagonism between these two elements has not been demonstrated (Pelletier 1985). Because many researchers have discovered high Hg levels in Odontoceti (toothed whales and dolphins), interpretation of the possible consequences should be made in light of the matching Se concentration. For example, Hg has been found at concentrations up to 485 $\mu g \cdot g^{-1}$ (wet wt) in liver tissue of apparently healthy striped dolphin (mean 205 $\mu g \cdot g^{-1}$, $n = 45$) (Honda et al. 1983). At this time, we do not know what concentration of mercury would be considered critical when in association with Se. Although this association is well defined in liver, it is not known if this antagonistic mechanism also occurs in other tissues such as brain.

Our main objective in this study was to examine several tissues for metals (Cd, Cu, Hg, and Pb) and metalloids (As and Se), determine their occurrence to assess accumulation, and search for correlations of these potentially toxic elements. Additionally, several mother-fetus pairs were examined in order to ascertain the degree of maternal transfer of these elements. We also attempted to relate the observed concentrations with known toxic effects in other mammals. Arsenic, Cd, Hg, and Se can be potentially very toxic to mammals but the relationship between their concentrations in tissues and toxic effects is unclear. We know of no studies that have examined captured cetaceans experimentally for lethal or sublethal responses to contaminants. Published studies on other mammals help us speculate about effects; however, most of that literature deals with administered dosage (e.g., micrograms per gram per day) which is information that is usually unavailable for whales. Pilot whales may be more or less sensitive than laboratory animals to the effects of certain metals, hence the comparisons made in this study are rudimentary.

Methods

Tissues were collected from beached animals (*Globicephala melaena*) during the month of December 1986 and 1990 on Cape Cod, MA. The 1990 animals stranded near Hyanisport, MA and the 1986 animals were from Eastham, MA, except for one animal from near Wellfleet, MA. Samples were collected and frozen shortly after autopsy by Greg Early (New England Aquarium, Boston, MA). The tissues were shipped on dry ice to our laboratory in Seattle, WA for analysis. We examined 5 female pilot whales from the 1986 stranding that included 4 fetuses. From the 1990 stranding, we examined 4 males and 11 females plus 4 fetuses. Because the ratio of dry to wet weight was very different for the liver samples of 3 whales from 1986, they were excluded due to concern over the state of degradation. The stomachs of animals examined in this study were empty of any recently ingested prey and the only contents observed were squid beaks.

Analytical Procedures

Tissues were prepared for atomic absorption spectroscopy by microwave digestion with high-purity nitric acid (SeaStar Chemicals, Sidney, British Columbia, Canada). Dry-wet weight ratios were obtained by weighing the tissue wet, freeze drying it in a model 12XL Freeze Dryer (Virtis ™, Gardner, NY), and then weighing the dried sample. We analyzed prepared digests for As, Cd, Cu, Hg, Pb, and Se. Arsenic, Cd, Cu, Pb, and Se were analyzed using heated graphite atomization atomic absorption spectroscopy with Zeeman background correction (Perkin-Elmer model 5100). A five-point standard curve was determined using solutions from single element Standard Reference Materials (SRM) (National Institute of Standards and Technology, U.S. (NIST)). Calibration was performed by a two-coefficient, least squares calibration line using the dedicated computer (Perkin-Elmer model 7000). Tissue concentrations are dry weights unless stated otherwise and are reported in micromoles per gram or micrograms per gram (ppm). Wet weight concentration can be determined by multiplying dry weight concentration by the quantity d/w found in Table 2. To determine micrograms per gram, multiply micromoles per gram by molecular weight of the element.

Total mercury content of tissue digests was determined by cold-vapor/gold amalgamation (CVGAAS) or cold-vapor (CVAAS) atomic absorption spectroscopy. Many samples were analyzed by CVGAAS because initially we believed that Hg concentrations would be low, especially in fetal tissue. This was not the case however, hence we switched to the less time consuming CVAAS method. To determine Hg with the amalgam system, 1 mL of sample or diluted sample was placed in the reaction chamber and stabilized by the addition of 2–3 drops of 5% aqueous potassium permanganate. The Hg vapor was liberated by the addition of 0.75 mL sodium borohydride reductant (3% $NaBH_4$ in 1% NaOH solution) with a syringe. The vapor was carried by an inert gas stream (argon, 100 $mL \cdot min^{-1}$) into a quartz tube containing high-purity gold foil. After a brief (1 min) purge, the carrier gas flow was stopped, and the quartz tube was heated with a coil of nichrome wire. After all Hg was released from the gold foil (90–120 s), the carrier gas flow was resumed, and the Hg vapor carried into the detection cell of the spectrophotometer (Perkin-Elmer model 5000). Spikes of 1, 2, 4, 6, and 8 ng Hg were used to obtain a linear calibration curve. Measurements from 38 calibration spikes indicated a mean and standard deviation (SD) sensitivity of 0.27 (0.03) ng.

Determination of Hg by the cold-vapor method was made by adding 1 mL of sample to a reaction cup containing 9 mL of 1.5% HC1 and approximately 5 drops of 5% aqueous potassium permanganate solution which was assembled on a Perkin-Elmer MHS-10 (Mercury/Hydride System). Reductant consisting of 3% $NaBH_4$ in 1% NaOH solution was directed into the cup by means of a manually operated valve. The mercury vapor generated was swept into a quartz cell connected to a Perkin-Elmer 5000 spectrophotometer and the manual valve was held open until a peak reading was observed. Calibration was determined with spikes of 5, 10, 20, 40, 80, and 160 ng Hg. Measurements from 24 calibration spikes yielded a mean (SD) sensitivity of 2.65 ng (0.16 ng).

Methyl Hg was analyzed by Brooks Rand, LTD, (Seattle, WA.) according to their standard operation procedure (S.O.P. BR0001). Samples were digested and ethylated to from volatile methyl ethylmercury. This compound was then chromatographed by cryogenic gas chromatography, pyrolytically broken down to elemental Hg, and detected using a cold vapor atomic fluorescence detector. Although there was no certified reference material (CRM) which contained a certified value for MeHg, Brooks Rand used DORM (National Research Council of Canada (NRCC)) which has

TABLE 1. Precision and Accuracy of Certified Reference Materials (CRMs). Precision is expressed with the coefficient of variation (CV) for 6–10 separate samples. Accuracy is expressed with the mean response factor (RF) which is the measured conc./CRM conc. RF mean, standard deviation (SD), and standard error of the mean (SEM) calculations based on all CRMs listed. Sample size is same for precision and accuracy. Hg† is CVGAAS (gold amalgam method) and Hg* is CVAAS method. See text for details. RF mean, SD, and SEM for Hg* based on replicates for DORM only ($n = 8$).

CRM	As	Cu	Cd	Hg†	Hg*	MeHg	Pb	Se
				Precision (CV%)				
DORM	22	23	41	8	22	5	24	11
DOLT	13	11	22	11	—	—	19	13
LUTS	45	16	9	14	—	—	—	18
1566A	24	7	19	24	—	—	19	29
TORT	26	—	6	2	—	—	15	33
Mean	26	14	19	12	22	5	19	21
n	42	36	42	25	8	6	36	37
				Accuracy (RF)				
Mean	0.27	0.79	1.0	1.5	0.67	0.98	0.81	0.39
SD	0.10	0.22	0.30	0.36	0.15		0.19	0.15
SEM	0.02	0.04	0.06	0.07	0.06		0.03	0.03

been analyzed repeatedly over a long period of time and has determined the mean (SD) MeHg to be 0.73 (0.06) $\mu g \cdot g^{-1}$ (dry wt).

We analyzed the CRMs: DOLT-1, LUTS-1, TORT-1, and DORM-1 (NRCC) and 1566a (NIST) concurrently and randomly with our samples in order to determine matrix interference, technique variability, and recovery efficiency which were used to calculate precision (repeatability) and accuracy (truth). We analyzed each element in 6–10 separate digested samples for each of the five different CRMs to assess our analytical error. To determine precision, a coefficient of variation (CV) was calculated for each CRM that was based on the mean and SD of the 6–10 measured concentrations (Table 1). Only DORM-1 was used for mercury (CVAAS) analyses because the concentration of Hg of all other CRM's was close to our limit of detection. Also listed in Table 1 is the mean, SD, and standard error of the mean (SEM) for the response factor (RF) (measured concentration/concentration of CRM) for each element which were calculated with all RFs for that element. Sample size for the response factor is the same as that used for precision (CV). The mean RF was used to adjust measured concentrations to reflect matrix interference and recovery loss. The limit of detection for each element in micrograms per gram dry weight was as follows: As, 0.1; Cd, 0.007; Cu, 0.1; Hg (CVGAAS), 0.006; Hg (CVAAS), 0.2; Pb, 0.05; and Se, 0.1.

To determine extractable lipid, an aliquot of a methylene chloride extract of tissue was filtered through filter paper containing approximately 5 g diatomaceous earth as a filtering aid and the solvent was removed from each sample using a rotary evaporator. After the solvent was removed, the mass of lipid was determined. The percentage lipid was determined by dividing the mass of lipid by the original sample wet weight and multiplying by 100. Using sea lion blubber ($n = 5$) and liver ($n = 5$) samples as test material this procedure yielded results for total lipids comparable to those obtained using the method of Hanson and Olley (1963), a modification of the Bligh and Dyer (1959) method. The mean (SD) percent total lipid (wet weight) in the sea lion blubber determined by our method and the Hanson and Olley method was 84(0.7) % and 80(2.2)%, respectively, and for the sea lion liver was 2.7(0.1)% and 2.2(0.2)%, respectively.

Statistical Analyses

Statistical analyses were performed with Statistica (StatSoft Inc. Tulsa, OK). Log_{10} transformed concentrations were used for all correlations and regressions. Pearson correlation coefficients were determined between elements in tissue and between elements and animal length. When sample size was small and one or two points influenced the significant correlation, the correlation was suspect. Also, when sample size was large ($n > 10$) and when one or two points had an undue influence on the significance of the correlation, we considered the linear correlation spurious. A *t*-test for independent samples was also performed to test for temporal differences in lipid levels in liver.

The residuals for all regressions (linear and non-linear) were checked to determine if the assumptions of the model (homoscedastic variances) were met. The non-linear model used for exploring the relationship between MeHg and Hg was an exponential decay function:

$$y = ae^{(nx)}$$

where y is the dependent variable (% MeHg), x is the independent variable (total Hg), and the coefficients a and n were estimated by an iterative non-linear algorithm. The loss function was $(\text{observed} - \text{predicted})^2$.

Standard deviations are reported to show the range in the data and the standard error of the mean is reported to indicate the precision of the estimate of the mean.

Results

In general, the concentrations of elements were quite variable between animals for a given tissue, which can be seen in the ranges reported (Table 2). The mean (SD) tissue concentration of each element was not different between adult male and female whales (except for brain Pb which was highest in males (0.21 $\mu g \cdot g^{-1}$ (0.18) $n = 3$; all females below detection limit

TABLE 2. Mean, standard deviation, and range of concentrations of elements in pilot whales from 1986 and 1990 strandings. Reported in μg/g dry weight. D:W = ratio of dry to wet weight, *n* = sample size, *only one above detection limit. Less than detection not included in mean.

	As				Cu				Cd				Hg			
	n	Mean	SD	Range	*n*	Mean	SD	Range	*n*	Mean	SD	Range	*n*	Mean	SD	Range
Adult																
Brain	7	1.0	0.6	0.5–2.2	7	9.1	2.3	5.7–12.3	7	0.4	0.4	<0.007–1.1	7	3.4	1.0	<0.2–4.4
Liver	7	1.8	0.5	1.3–2.6	7	15.5	3.6	9.9–20.3	17	35	29	0.05–131	17	176	201	1.9–626
Kidney	10	2.3	0.5	1.3–2.8	10	14.7	3.9	7.4–21.0	10	202	106	119–425	10	27.5	14.3	6.8–49.7
Ovary	4	1.7	0.7	0.8–2.2	4	5.5	2.4	2.8–8.4	4	3.1	2.6	0.13–5.9	4	65.1	81.1	1.9–173
Fetus																
Brain	3	1.4	0.8	0.5–2.1	3	5.1	1.0	4.4–6.2	3	0.02	0.002	0.018–0.020	3	1.3	0.5	0.7–1.7
Liver	8	0.8	0.3	0.3–1.2	8	97.7	53.9	15.3–166	8	0.02	*	<0.007–0.02	8	2.3	1.6	0.9–5.4
Kidney	3	1.1	0.6	0.6–1.8	3	20	10.5	8.1–28.1	3	0.64	0.5	0.07–1.1	3	1.9	1.7	0.6–3.9

	Pb				Se				MeHg				D:W		
	n	Mean	SD	Range	*n*	Mean	SD	Range	*n*	Mean	SD	Range	*n*	Mean	SD
Adult															
Brain	7	0.18	0.15	<0.05–0.41	7	3.0	0.9	1.4–4.5	3	1.7	0.9	1.1–2.7	7	0.19	0.04
Liver	17	0.41	0.26	0.05–0.91	17	128	190	2.3–695	3	8.0	2.2	5.6–10.0	17	0.27	0.03
Kidney	10	0.08	0.02	<0.05–0.11	10	14.3	7.8	3.9–32.9	1	2.9			10	0.26	0.04
Ovary	4	0.31	0.17	<0.05–0.42	4	28.7	44.1	3.4–94.4	1	1.0			4	0.24	0.003
Fetus															
Brain	3	0.19	0.03	<0.05–0.21	3	2.6	0.6	1.9–3.3	1	1.9			3	0.10	0.005
Liver	8	0.28	0.24	<0.05–0.52	8	2.5	0.8	1.6–3.8					8	0.20	0.06
Kidney	3	0.16	0.08	<0.05–0.21	3	2.8	0.3	2.5–3.1					3	0.13	0.01

except one individual = 0.11 $\mu g \cdot g^{-1}$, $n = 4$). Clearly in adults, elements such as Cd, Hg, and Se were quite high but variable between tissues (e.g., brain and liver). Conversely, mean As and Pb concentrations were less variable between tissues.

In comparing fetal and maternal tissues, mean concentrations of As, Cu, Hg, Pb, and Se were not greatly different in brain tissue, whereas Cd was much higher in maternal tissue (20 times). When fetal and maternal livers were compared, the mean concentration of As was the only element similar in both; whereas mean concentrations of Cd, Hg, and Se, were considerably higher in maternal tissue (1750, 77, and 51 times higher, respectively). As and Pb were relatively similar (within a factor of 2.3) and Cu was much higher (6.3 times) in fetal tissue. In maternal versus fetal kidney, mean As, Cu, Pb, were fairly similar (within a factor of 2), whereas mean concentrations of Cd, Hg, and Se were much higher in adults (315, 15, and 5 times, respectively). We also examined correlations of elements in the liver of matched fetal-maternal pairs ($n = 7$) from animals in the 1986 and 1990 strandings, but found none significant. Sample sizes were too small for other comparisons of maternal versus fetal tissue (kidney and brain).

Although only 3 fetal kidneys were available for analysis, the smallest fetus (64 cm) had the lowest As, Cd, Hg, and Pb concentrations. The smallest fetus also contained one of the lowest Hg concentrations and had non-detectable amounts of Cd and Pb in liver ($n = 8$; smallest = 64 cm, all others 90–105 cm).

Because the range in concentration was so large for some tissues, we searched for patterns that may have explained this variance. Several elements were correlated with animal length, which was a good surrogate for age (relatively linear for the lengths examined) (Sergeant 1962). The concentrations of Hg ($r = 0.94$) (Fig. 1a) and Pb ($r = 0.78$) (Fig. 1b) in the liver of adult stranded pilot whales were significantly correlated to animal length. Because most of the kidneys we analyzed were from large individuals (>400 cm) any significant correlation between length and element was obscured. In the case of liver Cd, it appeared that there was a threshold level of Cd associated with length that occurred around 225 cm (Fig. 1c); however, more data are needed to confirm this association.

Mercury was correlated with selenium in the liver of adults (Fig. 2), but not in kidney, brain, or ovary. The 1 to 1 molar ratio is plotted (Fig. 2) to indicate the level of protection against Hg toxicity. MeHg, as a percentage of total Hg, in different tissues of whales from 1986 and 1990 was inversely related to total Hg and can be described by an exponential decay function (Fig. 3). Total Hg was also significantly correlated with other elements found in the liver including Pb and Se and negatively with As (Table 3).

Other significant associations of elements occurred primarily in the adult liver that included selenium and lead (Table 3). Additionally, Pb and Cd in ovary were highly, but not significantly correlated ($r = 0.99$, $n = 3$) and all As correlations in adult liver were significantly negatively correlated. Most of the correlations listed in Table 3 are genuine in that the points are spread out along the regression line. However, all significant Cd correlations in adult liver, Cd and length (Fig. 1c), Cd and Pb ($r = 0.56$), Cd and Hg ($r = 0.77$), and Cd and Se ($r = 0.52$), appear to be spurious because only a few points determined the high linear correlation coefficient which was not representative of all the points in the regression (Table 3). Until more data are

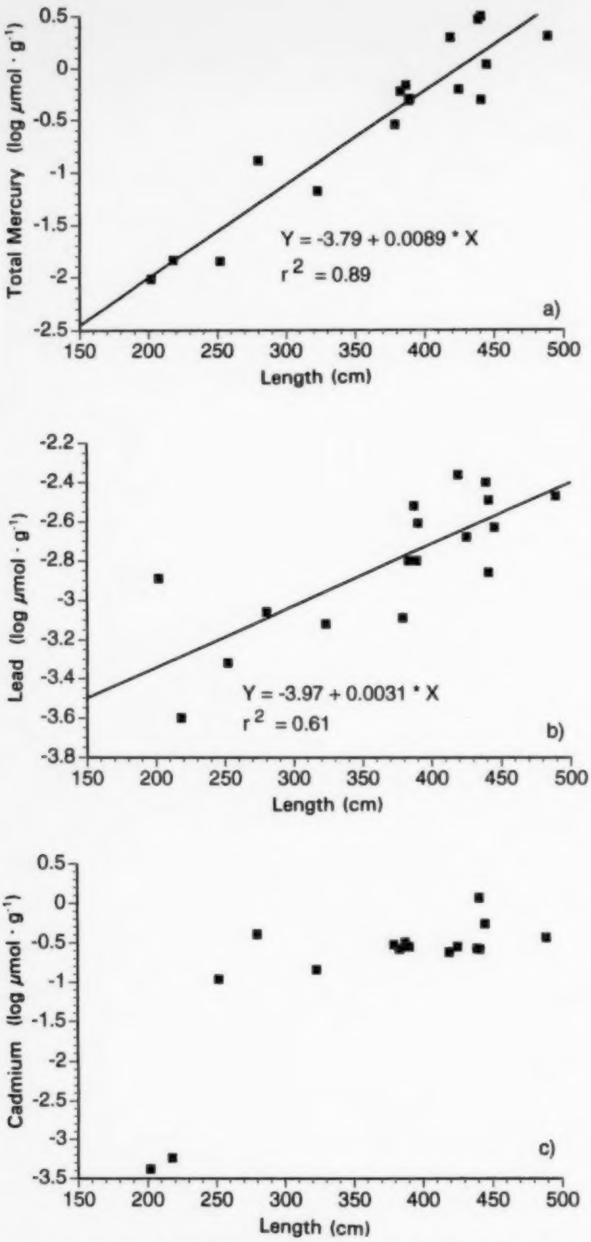

FIG. 1. Toxic metals in pilot whale tissue versus animal length. (a) Total mercury in adult liver; (b) lead in adult liver; (c) cadmium in adult liver.

available, we can conclude that all the significant linear correlations for Cd may not be genuine and that non-linear associations may exist.

The mean (SD) percent total lipid (wet weight) was 2.2(1.3)% ($n = 17$) in adult liver and 1.3(0.7)% ($n = 8$) in fetal liver. There was a significant difference in mean percent total lipid in fetal liver between the two different years. The mean (SD) value in fetal liver for 1986 was 1.9(0.3)% $n = 4$ and for 1990 was 0.7(0.2)% $n = 4$, $p = 0.0006$. The mean (SD) percent total lipid

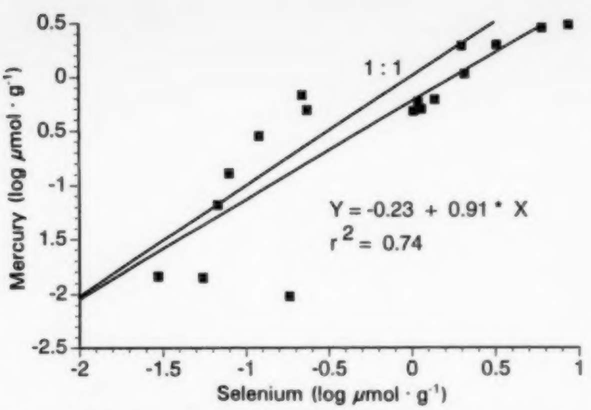

FIG. 2. Selenium and mercury in liver of adult pilot whale. 1:1 defines the line of equal molar amounts of Hg and Se.

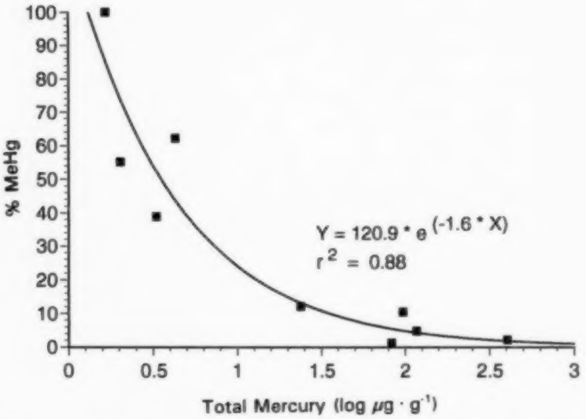

FIG. 3. Methyl mercury and total mercury in pilot whale tissues. Regression defines the association of percent methyl mercury (of total mercury) versus total mercury. Samples were from all tissues and included animals from 1986 and 1990 strandings. MeHg is methyl mercury.

TABLE 3. Pearson correlation coefficients (r) of elements in pilot whales from strandings. All correlations significant at $\alpha = 0.05$, except ovary Pb and Cd which was not significant. N is the sample size used in the correlation. All correlations calculated using log μmol · g dry wt^{-1}.

	Adult liver				
	Hg	Pb	Se	Length	*n*
As	−0.81	−0.83	−0.88	−0.78	7
Cd	0.77[a]	0.56[a]	0.52[a]	0.78[a]	17
Hg		0.85	0.86	0.94	17
Pb			0.80	0.78	17
Se				0.79	17

Other correlations	Pearson coefficient	Sample size
Pb and Cd in ovary	$r = 0.99$	3

[a] Denotes spurious linear correlations in which a few data points determine the association.

(1990 only) for fetal blubber was 7.0(8.7)% $n = 3$, and for adults was 75.7(8.5)% $n = 15$. There appeared to be no pattern between element profiles and lipid content.

Discussion

We analyzed elements which are thought to be potentially harmful to marine animals: four are non-essential elements (As, Cd, Hg, and Pb) whose abundance can be attributed to environmental exposure and two are essential (or required) elements (Cu and Se) whose abundance are controlled by physiological requirements in addition to environmental exposure. Because copper is an essential metal found naturally in high concentration (micromoles per gram), its abundance will change according to physiological need and homeostasis. Therefore its concentration in tissue is hard to interpret. As in our study, Julshamn et al. (1987) found the highest copper levels in the liver of pilot whale fetuses. Selenium is also an essential element, but is found naturally in low concentrations (1–5 $\mathrm{nmol \cdot g^{-1}}$) (Venugopal and Luckey 1978). In the present study, high concentrations of Se suggest that it has been bioaccumulated in excess of that required for normal function, and is probably related to high levels of Hg. When tissues such as liver and kidney were compared, especially between adult and fetus, the concentrations of some elements were much higher in adults and probably reflect exposure history. However, when brain was examined, there was little difference between these two life stages.

The significance of empty stomachs in this study is unclear. One possibility is that the animals were stressed before stranding which may have caused a cessation in feeding and a decline in health that lead to their beaching. Other possibilities include a loss of stomach contents due to the stress and physical hardship of stranding or loss due to continued digestion after stranding (Schwartz et al. 1992). Stoneburner (1978) also noted that stranded pilot whales (*Globicephala macrorhynchus*) had empty stomachs at the time of stranding and concluded that the whales may have been stressed and were using metabolic reserves. Because our data indicate that the lipid content of blubber was within normal limits (Henry and Best 1983; Martineau et al. 1987; Geraci 1989) the whales in our study were probably not using metabolic reserves at the time of stranding.

Maternal-Fetal Observations

We found no correlation between matched maternal and fetal liver for any of the elements studied. This may be due to the length of time needed to accumulate high levels of elements, the relative immobility of some elements (e.g., Hg and Se), and the fact that reproductively active females range in size (350 cm to over 500 cm) and have vastly different burdens of these elements. It is clear that some of the elements (As, Cd, Hg, Pb) examined were transferred from mother to fetus because they are not essential elements and hence in the absence of maternal transfer their tissue concentrations should have been below the limit of detection. Each of these elements is known to cross the mammalian placenta (Luckey and Venugopal 1977) (for review on Pb see Bell and Thomas 1980). The blood-brain barrier may be incomplete in whale fetuses, as it is in humans (Klaassen 1980), hence accumulation of these elements by fetuses may be unimpeded. Once the whales are independent, an effective blood-brain barrier and/or metabolic control may keep brain levels low allowing for the large difference between brain and liver concentrations.

Our data demonstrate transfer of Hg to fetuses. Mercury is particularly damaging to the brain and several studies have shown behavioral effects in small mammals (fed MeHg but total Hg measured) at about 2–10 $\mathrm{\mu g\ Hg \cdot g\ brain^{-1}}$ (wet wt) (=0.01–0.05 $\mathrm{\mu mol \cdot g^{-1}}$) (Fehling et al. 1975; Arito et al. 1982) and death at 15–30 $\mathrm{\mu g \cdot g^{-1}}$ (wet wt) (Wren 1986). Eccles and Annau (1982) demonstrated alterations in neonatal locomotor activity in rats when MeHg brain levels were 5–8 $\mathrm{\mu g \cdot g^{-1}}$ (wet wt). The concentrations of total Hg observed in pilot whale brain were below these concentrations and the 1:1 molar ratio. Because no correlation was observed between total Hg and Se in brain tissue, an antagonistic association of these two elements was not assumed.

Cadmium was much lower in liver and brain of developing whales, but when tissues are compared, Cd in fetal kidney was at least 30 times higher than Cd in the fetal liver. This supports Ronald et al. (1984) who concluded that Cd, Hg, Se, and Cu occur in seal pups through transplacental and/or transmammary transfer. In the present study, it appeared that Cd may be transferred to the fetus, which is in contrast to the conclusions of Fujise et al. (1988) for Dall's porpoise (*Phocoenoides dalli*). Additional support for maternal transfer in pilot whales comes from the differential accumulation of Cd observed in fetal kidney, a phenomenon which occurs in the adult.

Such transfer of toxic elements combined with the lack of blood-brain barrier may cause concentrations in the fetal brain to rise to levels that may result in developmental or behavioral abnormalities. Because prenatal and post-partum individuals could be more sensitive than adults to toxic contaminants, additional research should be done to assess possible toxicologic impairment. We are unsure if similar concentrations of As, Hg, Pb, and Se in maternal and fetal brain indicate that young whales start out with high concentrations of these elements with the potential for additional accumulation, or if this phenomenon reflects strong homeostatic control by adults. Adults must however acquire additional amounts of these elements in the brain as they grow because tissue concentrations would decrease through growth dilution if transfer to the brain was completely blocked.

Mercury in Tissues

The correlation between total Hg in pilot whale liver and animal length is very strong (Fig. 1a). Because adult length is a good surrogate measure for animal age (Sergeant 1962), the increasing concentration of total Hg indicates continuing exposure and accumulation over time. This linear correlation also implies a relatively constant imput of Hg, which presumably came from the whale's diet. Several studies have shown that squid, a major component of pilot whale diet (Sergeant 1962), can accumulate Hg in the 100–200 $\mathrm{ng \cdot g^{-1}}$ range (wet wt) (see Falandysz 1990 for a review).

In general, metals appear to bioaccumulate to higher levels in the predatory Odontoceti (toothed whales) than in the filter feeding Mysticeti (baleen whales). Mercury is the prime example here because it is known to biomagnify up the food web from herbivores to carnivores (Gardner et al. 1978; Wren 1986; Francesconi and Lenanton 1992). Mercury over 200 $\mathrm{\mu g \cdot g^{-1}}$ (1 $\mathrm{\mu mol \cdot g^{-1}}$) (wet wt) has been found in liver of dolphins and seals (Honda et al. 1983; Andre et al. 1991; Law et al. 1992) and is consistently high in the liver of pilot whales (Wagemann and Muir 1984; Muir et al. 1988). Conversely, Hg in the liver of baleen whales is relatively low, rarely exceeding 1 $\mu g \cdot g^{-1}$ (Johansen et al. 1980; Byrne et al. 1985; Honda et al. 1987; Varanasi et al. 1994). In this study, mean total Hg in the adult liver was 176 $\mu g \cdot g^{-1}$ (dry wt) (0.9 $\mu mol \cdot g^{-1}$) which is comparable to other studies of stranded (*G. macrorhynchus*) (Stoneburner 1978) and harvested (*G. melaenus*) (Julshamn et al. 1987) pilot whales. The levels in our study are also comparable to pilot whales (*G. macrorhynchus*) from a pristine area (St. Lucia, Lesser Antilles) (Gaskin et al. 1974). The authors of that study highlighted the importance of natural Hg sources, which are known to be high in that area.

The inverse correlation of %MeHg and total Hg is interesting because the %MeHg (of the total Hg) in fish, also a prey of pilot whales (Sergeant 1962), is generally very high (Bloom 1992; May et al. 1987). Because this relationship follows an exponential decay, it is similar to a first order toxicokinetic depuration model. Consequently, MeHg does not accumulate to high concentrations, but total Hg can achieve high levels which implies that demethylation is occurring in the animals by one or more mechanisms, including an innate mammalian pathway, a bacterial mediated process, or by an inorganic reaction. This is an advantageous feature for pilot whales because MeHg is considered more toxic than inorganic Hg (Venugopal and Luckey 1978) and the Hg may form an immobile complex with Se. This inverse relationship has been previously noted by Julshamn et al. (1987) and Falconer et al. (1983).

The high correlation between Hg and Se in the marine mammal liver has been noted before by several authors. In the present study the correlation was significant and 74% of the variation in the Hg data could be explained by the concentration of selenium (Fig. 2). It is interesting that the three lowest Hg values in this figure were from the smallest whales (<252 cm). Without the one obvious outlier, the correlation improved considerably ($r^2 = 0.83$). Other authors have shown a similar high correlation between these two elements in stranded (Stoneburner 1978) and harvested (Julshamn et al. 1987) pilot whales (*G. macrorhynchus* and *G. melaenus*, respectively) and other marine mammals (Kari and Kauranen 1978; Itano et al. 1984). It has been hypothesized that a complex is formed between these two elements that keeps them from their receptor(s) hence limiting induction of a toxic response (Naganuma and Imura 1980). In the present study a few of the whales displayed total Hg values that were greater than the protective 1:1 molar ratio (Fig. 2), but it is not known if this amount of excess Hg could cause a toxic response.

Cadmium in Tissues

Cadmium, which is known to accumulate preferentially in mammalian kidney, was very high in pilot whale kidney (mean 202 $\mu g \cdot g^{-1}$ dry wt) which is about 60% higher than that found in pilot whales (*G. macrorhynchus*) stranded in Georgia in 1977 (Stoneburner 1978) but essentially the same as that found in pilot whales (*G. melaenus*) harvested in 1978 in the Faroe Islands (between Iceland and Great Britain) (Julshamn et al. 1987), a relatively unpolluted area. The high Cd in pilot whale kidney could be due to a diet naturally high in Cd or from prey found in contaminated areas in which the whales may forage. Squid, a major component of pilot whale diet, can accumulate high concentrations of cadmium (Smith et al. 1984; Finger and Smith 1987). We believe that the concentrations of Cd observed in our study for pilot whales are some of the highest reported for marine mammals. The critical concentration of Cd in human kidney is believed to be in the range of 100–200 $\mu g \cdot g^{-1}$ (wet wt) (Nogawa et al. 1986) which is only about twice as high as the mean for our pilot whale kidneys (max about 100 $\mu g \cdot g$ wet wt^{-1}). Selenium is also known to reduce the toxicity of cadmium (Parizek et al. 1971) and may also be bound to Cd in a one-to-one molar ratio (Gasiewicz and Smith 1976, 1978). In pilot whale kidney, Cd was quite high and Se relatively low, hence little protection against toxicity was expected. It may be possible that in animals with high liver Cd, a Se–Cd complex will leave less Se available to complex and detoxify Hg.

Additional Element Correlations

Concentrations of lead in adult liver were also correlated with animal length (Fig. 1b) probably due to differential accumulation over time, which may explain the difference between adult and fetal values. The negative correlation between Pb and As (Table 3) is interesting, but occurs over a narrow range of arsenic concentrations. Selenium, Pb, Hg, and possibly Cd were all highly correlated with each other (Table 3) which was probably due to a long history of exposure. Cadmium and lead in whale ovary were also highly (but not significantly) correlated which may also be due to long-term accumulation.

Elements, such as Pb, Se, and As, may be toxic to mammals when levels are elevated. Studies with Pb have shown effects (clinical lead toxicosis) in cattle and horses (Osweiler and Van Gelder 1978) when concentrations are 18–26 $\mu g \cdot g^{-1}$ in the liver. Although pilot whales in this study exhibited increasing Pb in the liver with length (Fig. 1b), the levels in liver and kidney were not sufficiently high to warrant concern. When Pb levels in the brain are considered, it appears that some animals are more sensitive than others and juveniles and fetuses can be more sensitive than adults. Laboratory rats displayed altered behavioral activity when the concentration of Pb in the brain was in the range of 0.07–0.3 $\mu g \cdot g^{-1}$ (wet wt) (Cory-Slechta et al. 1983, 1985). These brain concentrations are approximately 3–10 times higher than the mean Pb concentration found in the stranded pilot whales, but overlap at the highest concentrations observed. Additional information from studies with mammals may help evaluate these concentrations in pilot whales.

Pilot whales have a large amount of Se in the brain (approx 3 $\mu g \cdot g^{-1}$), without the accompanying high Hg. We are unsure of the consequences of high Se levels in pilot whale brain: however, lethal levels of Se occur in mammals in the range of 1.5–10 $\mu g \cdot g$ body $weight^{-1}$ in laboratory animals after just a few days of exposure (Venugopal and Luckey 1978; Eisler 1985). Arsenic is toxic in cattle (Thatcher et al. 1985) and deer (NAS 1977) when concentrations in the liver are in the range of 14–19 $\mu g \cdot g^{-1}$ (wet wt), which is far above that observed for pilot whales in this study. Arsenic may also be a comutagen (Belton et al. 1985) and is a known teratogen (Luckey and Venugopal 1977).

In future studies of pilot whale strandings, we recommend continued analysis of the elements cadmium, lead, mercury and selenium in as many tissues as feasible in addition to pathological examination in order to assess possible toxic effects. More attention should be given to the Hg/Se interaction, especially in those tissues where the 1:1 molar ratio is not observed. Additional studies on the competition between Hg and Cd for Se would also be informative. We also recommend examining homeostatic control mechanisms, such as metallothionein, because when all toxic metals are considered together, such compensation mechanisms may be overwhelmed, and hence metal regulation lost, leading to toxic effects.

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Relative Importance of Microbes versus Macroinvertebrate Shredders in the Process of Leaf Decay in Lakes of Differing pH

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Tuchman, N.C. 1993. Relative importance of microbes versus macroinvertebrate shredders in the process of leaf decay in lakes of differing pH. Can. J. Fish. Aquat. Sci. 50: 2707–2712.

Four nonhumic lakes in northern Michigan, ranging in pH from 4.0 to 8.0, were selected to assess the effects of pH on leaf decay rates, leaf-associated macroinvertebrate assemblages, and the relative importance of microbes and shredders to the leaf decay process. Except for pH (and covariates of pH) these lakes were similar in physical and chemical parameters which directly affect metabolism. Preweighed leaves were placed in all four lakes for 8 wk; half of the leaves were confined in mesh bags to exclude shredders, while the others were not confined. Decay rates of confined leaves were not different among lakes, yet were lower than non-confined leaves in the alkaline lakes, suggesting shredders were important vectors of leaf decay in the alkaline lakes, but not in the acid lakes. Shredders comprised 65.9% of all invertebrates in Douglas Lake (pH = 8.0) and decreased in abundance (to 1.1%) with decreasing pH. Crustaceans and molluscs were few or absent in the acid lakes probably because of low pH and Ca^{2+} levels. Although microbial biomass on leaves appeared to be highest in acid lakes, estimates of microbial activity were significantly lower in the acid lakes, suggesting microbial metabolic inhibition at low pH.

Quatre lacs non humiques du nord du Michigan, dont le pH était compris entre 4,0 et 8,0, ont servi à l'évaluation des effets du pH sur la vitesse de décomposition des feuilles, sur les groupes de macroinvertébrés associés aux feuilles ainsi que sur le rôle relatif des micro-organismes et des déchiqueteurs dans le mécanisme de décomposition des feuilles. Exception faite du pH (et des covariables du pH), il y a entre ces lacs similitude des paramètres physico-chimiques qui ont un effet direct sur le métabolisme. Des feuilles pré-pesées ont été placées dans les quatre lacs pendant 8 sem. La moitié avait été logée dans des sacs-filets de manière à exclure les déchiqueteurs, l'autre moitié n'était pas confinée. La vitesse de décomposition des feuilles confinées était la même d'un lac à l'autre, mais elle était inférieure à celle des feuilles non confinées placées dans les lacs aux eaux alcalines (ce qui donne à penser que dans ces lacs, les déchiqueteurs constituent des vecteurs importants de la décomposition des feuilles), mais pas dans les lacs aux eaux acides. Les organismes déchiqueteurs correspondaient à 65,9 % de tous les invertébrés du lac Douglas (pH = 8,0); et plus le pH était bas, moins il y en avait (jusqu'à 1,1 %). Il y avait peu ou pas de crustacés et de mollusques dans les lacs acides, sans doute à cause du bas pH et de la faible concentration en ions Ca^{2+}. Même si la biomasse des micro-organismes semblait être la plus élevée sur les feuilles des lacs aux eaux acides, les évaluations de l'activité microbienne dans les lacs acides étaient significativement inférieures à celles des autres lacs; cela donne à penser que de bas pH inhibent le métabolisme microbien.

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Detrimental long-term effects of acidification on aquatic ecosystems have been documented in acid-sensitive regions of Europe (Leivestad et al. 1976; McCahon et al. 1989), Canada (Dermott et al. 1986), and the northeastern United States (Stevenson et al. 1985; Feldman and Connor 1992). The impacts include generally simplified food webs; reduced abundances and diversities of microbes (Osgood and Boylen 1992), macroinvertebrates (Dermott et al. 1986) and fishes (Beamish and Harvey 1972). Slow rates of leaf litter breakdown are also characteristic of acid lakes (Braekke 1976) and streams (Burton et al. 1985; Osgood and Boylen 1992). Whether the reduced utilization of this important organic carbon source (leaf litter) could be a result of direct pH toxicity to shredding invertebrates or insufficient shredder food sources due to reduced leaf-associated microbial assemblages is unclear (van Frankenhuyzen et al. 1985).

The purpose of this study was to investigate the process of summer leaf decay and leaf-associated decomposers in lakes of different pH in northern Michigan. Specific objectives included (1) assessing the relative importance of microbes (bacteria and fungi) and macroinvertebrates to the leaf decay process, (2) determining differences in leaf-associated macroinvertebrate assemblages, and (3) determining differences in microbial activities among lakes of different pH.

A preliminary survey of 21 softwater lakes in the poorly-buffered northern Michigan glacial shield was conducted to locate four study lakes whose predominant distinguishing physicochemical variable was pH. While lake morphometry was different among lakes, preliminary sampling indicated that these four lakes were nonhumic, contained fish, were not contaminated by heavy metals, and offered a wide pH range (Johnson Lake pH 4.0, Stump Lake pH 5.4, Piatt Lake pH 6.8, and Douglas Lake pH 8.0). Johnson and Stump Lakes had a conspicuous accumulation of intact leaves in the littoral zone, whereas amorphous black organic sediments dominated in alkaline Douglas Lake. Thus these lakes comprised a graded series of natural systems in

TABLE 1. Physical and morphometric measurements on four northern Michigan lakes. Data obtained from R. VandeKopple, UMBS, for Douglas Lake and from B. Luikkonen, USEPA Duluth, for the others.

Morphometric	Johnson	Stump	Piatt	Douglas
Latitude	46°N25′30″	46°N21′25″	46°N25′20″	45°N33′30″
Longitude	85°W02′40″	84°N52′30″	85°W00′30″	84°W40′28″
County	Chippewa	Chippewa	Chippewa	Cheboygan
Watershed (ha)	120.8	91.1	810.2	5650.0
Annual precip. (m)	0.7980	0.7980	0.7980	0.8214
Hydrologic type[a]	Seepage	Seepage	Seepage	Drainage
Maximum depth (m)	4.5	1.5	18.0	27.0
Mean depth (m)	2.97	0.5	5.09	5.50
Lake volume (m^3)	478 170	45 000	5 237 610	83 000 000
Renewal time (yr)	1.114	0.136	1.813	No data

[a] Seepage has no inlets or outlets. Drainage has both inlets and outlets.

which leaf decomposition appeared to be related to pH, and in which other chemical factors which influence leaf decay and associated biota did not vary among systems.

Leaf decay rates were monitored in the summer; much of the leaf litter enters these lakes in spring when snow melt and vernal rains flush litter into the systems via land overflow. Year-around leaf addition via inlet streams was either absent or minimal as Johnson, Stump and Piatt Lakes had no inlets and the creek entering Douglas Lake was small and transported little coarse particulate organic matter into the lake.

Materials and Methods

Morphometric and Physiochemical Characterization of the Lakes

An array of morphometric measurements were attained for all four lakes to assess general differences in lake size, location, watershed size, and drainage patterns (Table 1). Several physicochemical parameters were measured biweekly from 2 June to 2 August, 1985, to further characterize the study lakes. A Hydrolab Instrument Cluster (calibrated prior to each usage) was used to measure pH, dissolved oxygen, conductivity, and temperature. Whole water samples were collected in triplicate from the littoral zone of each lake and returned to the laboratory on ice where they were split into two parts. One part was titrametrically analyzed for total alkalinity and hardness (APHA 1989), and the second was filtered (0.45 μm Millipore) and frozen for later analyses of TP, PO_4^{3-}, NO_3^{2-}, NH_3, SiO_2, and Cl concentrations on a Technicon II Dual Channel Auto Analyzer. Copper, magnesium, and calcium concentrations were estimated with a Perkin-Elmer Model 303 Flame Atomic Absorption Spectrophotometer (APHA 1989) both in these water samples and on extracts of triplicate sediment samples collected with an Ekman dredge. Data for aluminum, iron, molybdenum, zinc, cobalt, manganese, lead, cadmium and nickel concentrations for these lakes were acquired from the USEPA National Water Quality Laboratory, Duluth, MN, and from the UMBS data bank.

Leaf Decay and Benthic Invertebrate Sampling

Leaf decay rates were estimated using dried (70°C), pre-leached *Acer saccharum* (sugar maple, a common riparian plant to all lakes) leaves, which were picked from a single tree during leaf abscission in the early winter of 1984. Each leaf was individually weighed, labeled, and tethered by the petiole to a brick with monofilament line. This method was employed rather than the construction of leaf packs commonly used for lotic systems (e.g., Petersen and Cummins 1974), to more accurately simulate the unconfined accumulation of leaves on the littoral shelves of lakes. To each brick, five individual leaves were tethered, as well as five leaves within a mesh bag (0.5-mm aperture) to exclude shredders, thereby distinguishing between shredder and microbial contributions to the process of leaf decay. Four assembled leaf-sets (a brick with 5 tethered and 5 bag-confined leaves) were placed in the littoral zone of each lake at depths ranging from 1.0 to 2.2 m on 2 June, and one leaf-set was collected every two weeks thereafter until 2 August, for estimates of summer leaf decay rates after 2, 4, 6, and 8 wk incubation. Bags were never found buried in sediment, or clogged with slit, and all leaves appeared to remain aerobic (none turned black or smelled of sulfur) throughout the 8-wk period. Upon collection, the individual leaves from both treatments (i.e., tethered and bagged) were washed thoroughly to remove any associated silt or sand, redried at 70°C, reweighed and averaged for an estimate of percent leaf dry weight remaining (*%R*). Percent remaining and an exponential coefficient of leaf decay (*k*) were calculated for each lake using methods of Petersen and Cummins (1974).

To assess assemblage structure of leaf-associated macroinvertebrates, a bottomless aluminum benthic invertebrate sampling box (0.21 m^2) was constructed so that the width of the box equaled the width of a commercial D-shaped dip net (31 cm). The box was placed randomly over naturally occurring leaf litter in the littoral zone of each lake at a depth that did not exceed the height of the box (75 cm), and all benthic invertebrates and leaves within the sampler were removed with successive sweeps of a dip net (0.5 mm aperture). This method proved to be >96% efficient at collecting all invertebrates >0.5 mm that occurred within the sampler box (as measured by siphoning all remaining contents of the benthic invertebrate sampling box through a 0.5-mm soil sieve and examining all organisms missed by the dip net). Invertebrates in three samples from each lake were identified to species (or lowest feasible taxa), enumerated, assigned to functional groups (Merritt and Cummings 1984), and Shannon's diversity and taxa richness were calculated for the assemblages in each lake.

Assessment of Microbial Activity

To quantitatively estimate the microbial activity on leaf litter, additional sugar maple leaves enclosed in 0.5-mm mesh bags were placed in each lake and incubated for 4 wk in July.

TABLE 2. General physicochemical parameters (mean ± SD) of four northern Michigan lakes from biweekly samples collected between May and August ($n = 11$ for each parameter).

Parameter	Johnson	Stump	Piatt	Douglas
pH	4.0 (0.3)	5.4 (0.4)	6.8 (0.3)	8.0 (0.4)
Conductivity (μS)	7.4 (9.2)	12.5 (11.1)	64.7 (7.9)	235 (11.7)
Alkalinity ($mg \cdot L^{-1}$)[a]	0.5 (0.5)	6.5 (0.5)	35.3 (0.3)	121.5 (0.5)
Hardness ($mg \cdot L^{-1}$)[a]	4 (0)	12 (0)	40 (0)	132 (0)
H_2O-column Ca^{2+} ($mg \cdot L^{-1}$)	0.28 (0.48)	1.00 (0.67)	4.61 (2.31)	26.9 (0.69)
Sediment Ca^{2+} ($\mu g \cdot g^{-1}$)	0 (0)	48 (63)	65 (20)	1074 (445)
H_2O-column Mg^{2+} ($mg \cdot L^{-1}$)	0.08 (0.04)	0.41 (0.05)	3.60 (0.09)	11.4 (0.13)
Sediment Mg^{2+} ($\mu g \cdot g^{-1}$)	7.2 (0.5)	20.3 (13.4)	22.8 (2.4)	84.9 (30.3)

[a] As $CaCO_3$ milli equivalents.

TABLE 3. Average leaf decay constants (k) ($n = 5$) expressed as per-day rates. Standard errors were based on the exponential decay model for leaf weight loss. Also presented are average leaf weight remaining (%R) values for the last day of the study (wk 8) for all lakes.

Lake	Tethered Leaves			Bagged
	k (d^{-1})	SE	%R (8 wk)	%R (8 wk)
Johnson (acid)	0.00607	0.00070	42.4	51.4
Stump (acid)	0.00685	0.00092	47.2	47.6
Piatt (neutral)	0.01627	0.00620	17.0	43.2
Douglas (alkaline)	0.02092	0.00967	15.3	40.9

Microbial activity on these leaves was assayed by placing twelve 1.0 cm diameter leaf discs in each of three opaque biological oxygen demand (BOD) bottles which were filled with 300-mL filtered (0.45-μm) water from the corresponding lake and gently circulated on a magnetic stir plate. Changes in dissolved oxygen over a 5-d period at 18°C were monitored every 8 h using a YSI DO probe (designed to fit a BOD bottle and calibrated by Winkler titrations). Microbial activity was reported as micrograms O_2 consumed per square centimetre of leaf tissue per hour. Using the same technique, oxygen uptake (via microbial activity) of unfiltered littoral water was estimated for each lake).

Statistical Analyses

Pearson correlations of pH (as H^+ ion concentration) with other physicochemical and biological parameters were made using the general linear models (GLM) procedure (SAS 1989). Leaf decay rates and microbial activity among lakes were compared using a 1-way ANOVA followed by a Student-Newman-Keuls (SNK) multiple comparison (SAS 1989) at the 0.05 level. Shannon's Diversity indicies (Vandermeer 1981) were calculated for benthic macroinvertebrate assemblages, and taxa richness was determined by averaging the total number of different macroinvertebrate taxa enumerated from three samples for each lake.

Results

Physicochemical parameters that were significantly positively correlated with pH (GLM; conductivity $r^2 = 0.86$, $p < 0.001$; alkalinity $r^2 = 0.90$, $p < 0.001$; hardness $r^2 = 0.81$, $p < 0.001$; Ca^{2+} $r^2 = 0.92$, $p < 0.001$; Mg^{2+} $r^2 = 0.78$, $p < 0.001$) differed considerably among lakes (Table 2). Nutrient levels were generally low and similar among lakes with PO_4^{3-} being the limiting nutrient in all lakes, while DO and temperature measured at the sediment-water interface in the littoral zones were moderately high and similar among lakes (DO was always saturated, and average temperatures ranged from 18.5 to 22.0°C). Heavy metal concentrations were very low or not detectable in all lakes, with no relation to pH (GLM; $r < 0.44$ and $p > 0.05$ for all correlations of metals with pH).

Leaf decay rates in the absence of shredders (bag-confined leaves) were not significantly different among lakes (SNK; $p > 0.05$, Table 3, Fig. 1a), whereas tethered leaves decayed significantly faster in the alkaline and circumneutral lakes than in the two acid lakes (SNK; $p < 0.05$; Table 3; Fig. 1b). The alkaline and circumneutral lakes had significantly higher shredder biomass than the acid lakes (t-tests; $p < 0.05$; Fig. 2). Further, weight loss of tethered, Douglas and Piatt Lake leaves was higher (t-tests; $p < 0.05$) than shredder-excluded leaves within mesh bags for those lakes. Johnson and Stump lakes showed no difference (t-tests; $p < 0.05$) in %R between tethered and bag-confined leaves.

A comparison of total benthic invertebrate functional group composition (percent biomass) among lakes indicated that shredders were significantly lower in low pH lakes, comprising 1.1% of the invertebrates in Johnson Lake and 65.9% in Douglas Lake (GLM; $p = 0.05$, $r^2 = 0.88$; Fig. 2); these differences were also significant for actual abundance data (GLM; $p = 0.05$, $r^2 = 0.79$). Grazers were also lower in acid lakes, but the trend was not significant, whereas abundances (percent biomass) of predators and collectors were significantly higher with decreasing pH (GLM; $p = 0.05$, $r^2 = 0.81$ and $r^2 = 0.76$, respectively) (Fig. 2). While total littoral benthic invertebrate densities varied widely among lakes (Johnson = $1090 \cdot m^{-2}$, Stump = $377 \cdot m^{-2}$, Piatt = $199 \cdot m^{-2}$, Douglas = $1244 \cdot m^{-2}$), total invertebrate biomass was significantly higher in lakes having pH > 7.0 (Piatt = $288\ mg \cdot m^{-2}$, Douglas = $331\ mg \cdot m^{-2}$) than

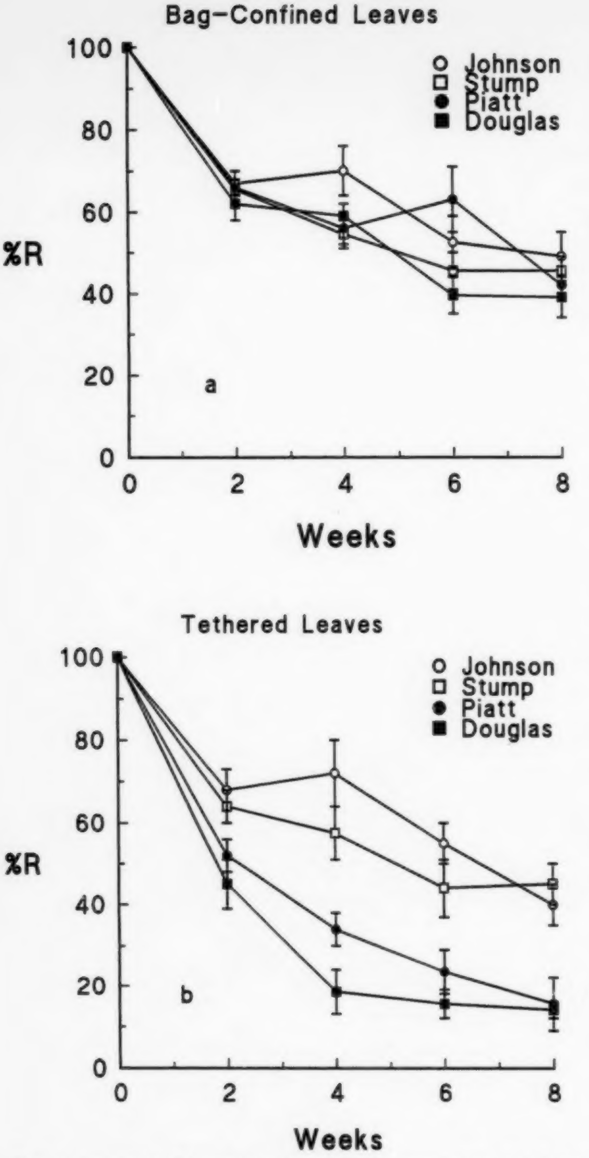

FIG. 1. Average leaf decay ($n = 5$) (±1 SD) expressed as % dry leaf weight remaining (%*R*) over time in all lakes for (a) bag-confined and (b) tethered leaves.

in lakes of pH < 5.5 (Johnson = 225 mg · m^{-2}, Stump = 201 mg · m^{-2}) (*t*-tests; $p < 0.05$). Both Shannon's diversity and taxa richness were lower in the acid lakes than in Piatt and Douglas lakes (Table 4). The above mentioned differences can be explained by differences in the invertebrate taxa that dominate within the alkaline and acid lakes. The greatest difference among invertebrate assemblages was observed within the order Diptera, which accounted for 6.2 and 17.5% of all invertebrates (by density) in Douglas and Piatt lakes, respectively, while dominating in Stump and Johnson lakes, accounting for 71.0 and 69.8%, respectively (Table 4). Within the shredder guild, crustaceans dominated in alkaline lakes while a non-crustacean dominance was observed in the acid lakes. In Douglas and Piatt lakes, isopods (*Caecidotea* sp.) and amphipods (*Gammarus limnaeus*) were the dominant shredders, while these crustacean groups were either under-represented or not present at Stump and Johnson lakes, and >95% of the shredders here were chironomids (mostly *Polypedilum fallax).* These differences in taxonomic groups strongly correlate with Ca^{2+} levels (GLM; $p = 0.05$, $r^2 = 0.92$), which may be related to high Ca^{2+} requirements of crustacean exoskeletons. Among the other functional groups, taxonomic differences between alkaline and acid lakes included fewer Mollusca and Trichoptera and greater abundances of Chironomidae and Odonata in acid lakes (Table 4).

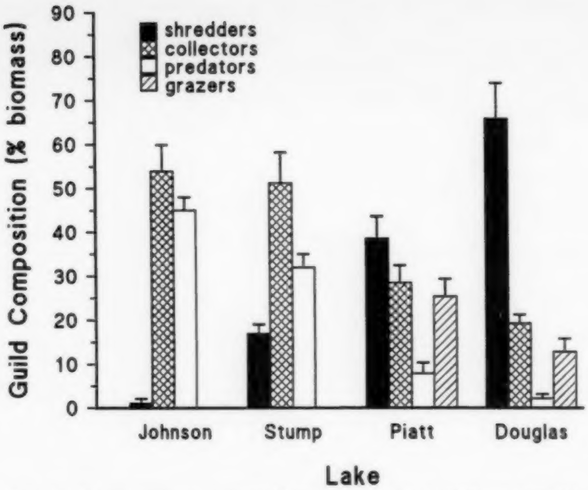

FIG. 2. Mean percent composition ($n = 3$) (+1 SD) of benthic macroinvertebrate functional guilds estimated by biomass for each lake.

Microbial activity on leaf discs and in the water column was not related to pH (GLM; $p = > 0.05$, $r^2 = 0.33$). However, mean respiration rates (averages of leaf disc activity and water column activity) in Douglas Lake were significantly higher than all other lakes (SNK; $p < 0.05$), while Stump Lake respiration rates were higher than in Johnson Lake (SNK; $p < 0.05$), and not different from Piatt Lake (SNK; $p > 0.05$) (Fig. 3). Within each lake, the relative magnitudes of microbial activity on leaf litter (as micrograms O_2 consumed per square centimetre per hour) and microbial activity in the water column (as micrograms O_2 consumed per litre per hour) were consistent (Fig. 3).

Discussion

Observed differences in leaf decay rates, macroinvertebrate functional group composition, and microbial respiration rates among lakes were likely a function of pH and its chemical correlates (e.g., alkalinity, Ca^{2+}, Mg^{2+}, conductivity, and hardness), since other major physicochemical parameters were similar among lakes. However, while a number of patterns could be attributable to pH in the following discussion, it is recognized that pH may directly or indirectly be responsible for only some observed effects and that other potentially important factors may be acting on the leaf decay process simultaneously. Common responses to acidification such as increases in heavy metal concentration and loss of fish fauna (e.g., Holtze and Hutchinson 1989; McCahon et al. 1989) were not associated with the pH gradient investigated here (personal observation).

TABLE 4. Average composition of benthic invertebrate communities ($n = 3$) by major taxonomic groups as % by density (n) and % by biomass (m). Also reported are average Shannon's diversity indices and taxa richness for all lakes.

Taxa		Johnson	Stump	Piatt	Douglas
Amphipoda	n	0	7.2	38.4	55.6
	m	0	5.9	37.2	48.0
Diptera	n	69.8	71.0	17.5	6.2
	m	42.4	52.0	6.0	1.4
Mollusca	n	0	0	11.4	13.4
	m^a	0	0	28.5	23.0
Trichoptera	n	2.9	0.6	15.5	14.6
	m^a	9.8	1.8	11.2	12.2
Coleoptera	n	26.5	13.3	3.4	8.2
	m	16.3	5.1	3.7	4.4
Odonata	n	0.8	1.8	1.7	0.1
	m	30.4	32.0	6.3	7.2
Others	n	0.5	2.4	0	0.5
	m	0.1	1.3	0	0.4
Shannon's diversity		2.2	1.9	3.0	3.4
Taxa richness		10.0	11.3	18.0	20.3

[a] Soft tissue biomass only; shells and cases excluded.

Decay rates for non-confined leaves were slower in acid than in non-acid lakes in this study and others (e.g., Burton et al. 1985). Per-day decay coefficients (k) for non-confined leaves in the acid lakes (0.00607 and 0.00685) were comparable to those of *Acer saccharum* in five acid systems of similar pH in the Adirondacks reported by Osgood and Boylen (1992), which ranged from 0.00133 to 0.00709, but were significantly lower than those for the alkaline and circum-neutral lakes. These experimental results were consistent with observations of build-up of undergraded leaves on littoral shelves of Stump and Johnson lakes. Quantitative littoral benthic samples revealed a paucity of shredders in the acid lakes, and no difference in decay rates was measured between leaves in bags (where shredders would be excluded), and non-confined leaves (where both microbes and shredders had access to colonize). Yet, in the alkaline and circum-neutral lakes, shredder abundance was greater resulting in significantly higher rates of leaf decomposition for tethered leaves as compared with shredder excluded (bag-confined) leaves. These results suggest that the microbial components of all lakes had similar effects on the rates of leaf decay, but since the acid lakes did not support shredders, tethered leaves in the acid lakes decomposed at the same rate as shredder-excluded leaves in the acid lakes (while tethered leaves in the alkaline lakes decayed faster).

Not only were shredders scarce in the acid lakes, but they were represented only by chironomidae, whereas crustacean shredders were predominant in Douglas and Piatt lakes. Bradt and Dudley (1986) reported similar patterns in numerical dominance from Amphipoda in alkaline to Chironomidae in acid lakes of Pennsylvania. Chironomids accounted for up to 63.1% of benthic invertebrates in a survey of 6 acid lakes (pH 5.7–6.0) in Ontario (Dermott et al. 1986), while molluscs and crustacean were considered to be relatively sensitive to low pH (Okland 1969; Sutcliffe and Marrick 1973). Similarly, molluscs, isopods, amphipods and decapods were scarce or absent in the acid lakes of the present study, yet abundant in Douglas and Piatt lakes. Low calcium levels in the acid lakes may partially explain the absence of these invertebrate groups. The requirement of calcium for the shells or carapaces of these organisms is well known; for example Wright (1980) describes the critical Ca^{2+} requirements (0.1 mM) in tissues of an actively moulting *Gammarus pulex*.

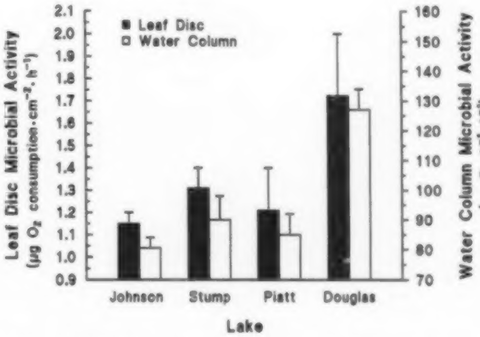

FIG. 3. Mean microbial activity (O_2 consumption rate) ($n = 3$) (+1 SD) measured from leaf litter and the water column for each lake.

Evidence in the literature suggests that the absence of certain shredders in acid systems is most likely a function of pH-toxicity, and not food quality/quantity of leaf litter and its associated microbes. In a study by van Frankenhuyzen et al. (1985), early instars of a shredding caddisfly experienced low survival at low pH due to acid toxicity, while late instar survivors grew faster on leaf litter conditioned at low pH than on leaf litter conditioned in alkaline systems. It is plausible that the young life cycle stages of crustaceans which frequently moult could be affected by low pH and/or the lack of Ca^{2+}. Allan and Burton (1986) reported size-dependent sensitivity of *Asellus intermedius* and two other stream invertebrates to laboratory depression of pH, where eggs and smaller instars were totally eliminated at pH 4.0. Further, they reported survival at low pH of isopods larger than a size threshold of 0.5 mg dry weight. Similar responses have been reported for other crustaceans including amphipods (France and Stokes 1987), eubranchiopods (Borgstrom and Hendrey 1976), cladocerans and copepods (Bruns and Wiersma 1988), and decapods (Davies 1989). In the present study, no isopods, amphipods, decapods or molluscs were found to occur in lakes having a pH < 5.5 and low (or no) Ca^{2+}.

Chamier (1987) suggested that although relatively large microbial populations can exist in acid systems, their metabolism appears to be inhibited, leading to low rates of decay. Allard and Moreau (1986) measured no qualitative differences in C:N ratios of leaves incubated in streams of pH 4.0 versus pH 6.2–7.0, indicating microbial biomass was not lower in acid streams. Bacterial activities (measured as ATP) on *Acer saccharum* were low and not related to pH in acid systems in the Adirondacks (Osgood and Boylen 1992). In the present study, flocculent "mats" of fungi observed on the benthic surface of Johnson Lake, in particular, suggested that microbial biomass was high, yet microbial activity (O_2 uptake) measured on leaf packs and in the water column was lower in Johnson, Stump, and Piatt than in Douglas Lake.

Simplified food webs and reduced biotic diversity have been found to be a consequence of low pH stress in acid systems (Beamish and Harvey 1972; Dermott et al. 1986; Osgood and Boylen 1992); similar observations were made in this study. While the littoral zone of Douglas Lake supports algal-based and detrital-based food webs which are highly productive and support several trophic levels, the acid lakes in this study lacked trophic components from both food webs; detritus was plentiful but detritivores were missing, and grazers were missing from algal-based food webs. Mats of benthic algae were observed in the acid lakes (particularly Stump Lake), while "clouds" of filamentous *Mougeotia* sp. (Chlorophyta) floated throughout the limnetic zone in Johnson Lake. Similar occurrences of *Mougeotia* have been reported in other acid lakes (Muller 1980, Stevenson et al. 1985). Planas et al. (1989) describes an increase in periphytic biomass in acidified experimental troughs and attributes it to the decrease in grazers. Muller (1980) showed that although the rates of carbon fixation were lower at pH 4.0, standing crops of periphytic algae were greater since grazers were absent. Both the abundant littoral leaf litter and algae seemed to provide niches that were under-utilized in these lakes, suggesting the stress of pH toxicity may be great enough to eliminate certain trophic positions, possibly resulting in less efficient energy transfer through the food webs.

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Composition and Sedimentation of Aquatic Pigments Associated with Deep Plankton in Lakes

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High Performance Liquid Chromatography (HPLC) pigment techniques were applied to study planktonic pigment composition and sedimentation in four Wisconsin (USA) lakes. In each lake, deep blooms of phytoplankton overlaid phototrophic sulfur bacteria in the region of the oxic-anoxic interface. Chlorophyll *a* and bacteriochlorophyll (*a, d,* and *e*) were observed as indicators of phytoplankton and phototrophic sulfur bacteria, respectively. Major taxonomic classes of phytoplankton were further resolved based on carotenoid signatures. In contrast to three oligo-mesotrophic Wisconsin lakes in which deep phytoplankton were comprised of dinoflagellates (indicated by the carotenoids peridinin, diadinoxanthin), cryptophytes (alloxanthin), and chrysophytes (fucoxanthin), the deep phytoplankton community in mesotrophic Mirror Lake was dominated by blue-green algae (oscillaxanthin, myxoxanthophyll, zeaxanthin, and echinenone). Deep planktonic production strongly influenced sedimentation of algal pigments and obscured the relationship between apparent water quality and sedimentary pigment deposition.

Les techniques d'analyse des pigments par chromatographie liquide à haute performance (CLHP) ont été appliquées à l'étude de la composition et de la sédimentation des pigments planctoniques dans quatre lacs du Wisconsin (É.-U.) Dans chaque lac, d'épaisses proliférations phytoplanctoniques recouvraient les bactéries sulfo-réductrices qui se trouvaient à l'interface de la zone oxique et de la zone anoxique. On a constaté que la chlorophylle *a* et que la bactériochlorophylle (*a, d* et *e*) sont des indicateurs du phytoplancton et des bactéries sulfo-réductrices, respectivement. Les grandes classes taxonomiques de phytoplancton ont été identifiées de manière plus poussée à partir de la signature que constitue leurs caroténoïdes. Par comparaison aux trois lacs oligo-mésotrophes du Wisconsin où le phytoplancton profond se composait de dinoflagellés (dont la présence était indiquée par des caroténoïdes, la péridinine et la diadinoxianthine), des cryptophytes (alloxanthine) et des chrysophytes (fucoxanthine), alors que la communauté phytoplanctonique profonde du lac Mirror, mésotrophe, était dominée par les algues bleues (oscillaxanthine, myxoxanthophylle, zéaxanthine et échinénone). La production de plancton profond influence considérablement la sédimentation des pigments algaires et masque la relation entre la qualité apparente de l'eau et le dépôt de pigment sédimentaire.

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Assessment of water quality or production characteristics of lakes by sampling surface waters and extrapolating the results to the entire water column lacks validity for many lakes. Uniform algal biomass (chlorophyll) profiles are usually only found in shallow, well-mixed systems. In contrast, many lake (Eberly 1959; Ichimura et al. 1968; Baker and Brook 1971; Fee 1976; Pick et al. 1984; Fahnensteil and Scavia 1987; Shafer 1988; Konopka 1989) and oceanic (Steele 1964; Anderson 1968; Venrick et al. 1973; Gieskes et al. 1978; Veldhuis and Kraay 1990) systems possess deep chlorophyll maxima, undetected by surface sampling. In addition to subsurface chlorophyll peaks, numerous lakes may contain large populations of phototrophic bacteria in anoxic waters (Sorokin 1965; Czeczuga 1968; Takahashi and Ichimura 1968; Culver and Brunskill 1969; Cohen et al. 1977; Lawrence et al. 1978; Parkin and Brock 1980; Hurley and Watras 1991). These bacteria are obligate anaerobes usually found at depths where anaerobic conditions persist and nutrients and light are available for growth. In some lakes, such as Fayetteville Green Lake, phototrophic bacteria can contribute a significant portion (83%) of the annual primary production (Culver and Brunskill 1969). In contrast, Parkin and Brock (1980) found that photosynthetic sulfur bacteria accounted for between 0.26 and 6.3% of the daily production of six Wisconsin and Michigan lakes.

The presence of phototrophic bacterial layers in lakes has previously been ascribed to lakes with rather atypical conditions, such as meromictic Lake Ciso (Guerrero et al. 1985; Pedros-Alio et al. 1986, 1987), a deep, small surface area lake; Lake Vechten

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TABLE 1. General physical and chemical characteristics of study lakes. Data for northern Wisconsin lakes from Mercury in Temperate Lakes Project (C.J. Watras, principal investigator). Chemical characteristics are based on spring overturn. ANC = acid neutralizing capacity ($\mu eq \cdot L^{-1}$). DOC = dissolved organic carbon ($mg \cdot L^{-1}$). Total P = total unfiltered phosphorus ($\mu g \cdot L^{-1}$). Trophic status based on phosphorus levels at spring overturn.

Lake	Surface area (ha)	z_{max} (m)	z_{mean} (m)	pH	ANC	DOC	Total P	Trophic status
Little Rock Treatment	9.8	10.3	3.8	5.0	−4.5	1.1	12	Oligo-mesotrophic
Pallette	70	18.2	9.6	7.0	127	5.3	14	Oligo-mesotrophic
Russet	19	11.0	2.9	5.8	27	6.8	14	Oligo-mesotrophic
Mirror	5.1	13.1	7.8	8.2	3300	N/A	31	Mesotrophic

(Steenbergen and Korthals 1988); or a farm pond, Knaack Pond (Parkin and Brock 1980). However, in a study of two oligotrophic seepage lakes in northern Wisconsin, Hurley and Watras (1991) identified deep chlorophyll maxima directly above bacteriochlorophyll from phototrophic sulfur bacteria. Similarly, in a study of 49 reservoirs in the Midwest, Knowlton and Jones (1989) found that nine of the reservoirs contained deep bacterial layers. Although spectrophotometric techniques with their recognized limitations were used, the authors identified the importance of phototrophic bacteria in these reservoirs while stressing the need for better pigment-specific techniques (such as HPLC) to confirm their observations.

Deep chl and Bchl maxima in Wisconsin lakes mainly occur at or below the thermocline region (Garrison and Knauer 1981; Hurley and Watras 1991; LTER 1992). The transition zone between oxic and anoxic conditions usually represents a zone of intense chemical gradients, and in many Wisconsin lakes, this zone often contains strong gradients of redox-controlled elements such as iron, sulfur, and phosphorus (Juday et al. 1938; Stauffer 1974; Hurley 1984; LTER 1992). Lakes that develop anoxic hypolimnia and have adequate levels of light and sulfide are favorable sites for phototrophic bacterial production (Madigan 1988). Under suitable conditions, both algae and phototrophic bacteria may be present in these transition zones in lakes.

The use of High Performance Liquid Chromatography (HPLC) is rapidly becoming the preferred technique for identification and quantification of algal pigments (Mantoura and Llewellyn 1983; Bidigare et al. 1985; Wright et al. 1991). Estimation of chl and chl degradation products (chlorophyllide, pheophytin, pheophorbide), together with individual carotenoids specific to certain algal groups, yield substantially greater information than traditional absorbance or fluorometric chlorophyll assays (Holm-Hansen et al. 1965; Lorenzen 1967; APHA 1989). HPLC techniques also have been used successfully to quantify pigments associated with phototrophic sulfur bacteria in lakes (Korthals and Steenbergen 1985; Steenbergen and Korthals 1988; Hurley and Watras 1991). Interferences inherent with previous chlorophyll *a* (chl *a*) techniques, such as the overlapping spectral properties of bacteriochlorophyll *d* (Bchl d) and chl *a* while using spectrophotometric estimation techniques (Caraco and Puccoon 1986; Knowlton et al. 1989), are resolved by the use of HPLC (Hurley and Watras 1991). This additional information on specific chlorophylls, coupled with detection and quantification of individual carotenoids, has led to numerous HPLC applications in oceanographic and limnological studies of water column plankton dynamics (Bidigare et al. 1986; Gieskes and Kraay 1986; Hooks et al. 1988; Repeta et al. 1989; Hurley and Armstrong 1990; Repeta and Simpson 1991), trophic transport (Carpenter et al. 1986, 1988; Leavitt et al. 1989; Storm and Welschmeyer 1991), and sedimentary diagenesis (Repeta and Gagosian 1987; Repeta 1989; Hurley et al. 1992).

In this study, we used HPLC techniques to evaluate the pigment composition in four Wisconsin lakes that possess characteristics amenable to the formation of deep plankton layers. Through analysis of these important biomarker pigments (individual chls, Bchls, and carotenoids), we examined the distribution of pigments associated with phytoplankton and bacterioplankton in the water column of lakes with contrasting depth and trophic status. Secondly, we assessed the importance of deep plankton in dictating pigment sedimentation of these contrasting lakes. Deep planktonic production may also complicate paleolimnological interpretation of pigment stratigraphies of bottom sediments. Frequently, residual pigment remnants in sediments are used to estimate past trends in overall water quality (Swain 1985; Zullig 1989). If pigment sedimentation is predominantly influenced by sedimentation from deep layers, the reliability of interpretations of "apparent" (surface) water quality derived from pigment stratigraphy may be weakened.

Methodologies

Study Site

Four lakes in central and northern Wisconsin were chosen for study (Table 1). Three remote lakes in glacial outwash of northern Wisconsin — Pallette (46°04′N, 89°42′W), Russet (46°11′N, 89°36′W), and Little Rock Lake (Treatment Basin, 45°50′N, 89°36′°W) — were sampled as part of a larger program on mercury cycling (Watras et al. 1991). Water column and sediment trap sampling schedules reflected overall goals of that project. A fourth lake, is also located in glacial outwash. Mirror Lake (44°21′N, 89°05′W) in central Wisconsin was the site of a demonstration project in which storm sewer outfalls were diverted from the basin in 1976. In 1978, aluminum sulfate was added to precipitate residual phosphorus from the water column and to retard phosphorus release from bottom sediments (Garrison and Knauer 1983, 1984). Intensive water column and sediment trap sampling was conducted in 1990 as a phase of a project dealing with assessment of the effect of mitigation on long-term lake recovery. All four lakes each developed strong thermal stratification and hypolimnetic oxygen depletion in the late summer and early fall.

Field Sampling

Samples for pigment analysis were collected by in-line filtration at selected depth intervals, with greater detail in the region of deep planktonic production. Approximately 1–2 L of water was filtered through 47 mm GF/F filters housed in Nuclepore filter holders. Filters were placed in the dark on ice and transported to the laboratory for extraction. If filters were stored prior to extraction (at most 1–3 d), they were frozen at −10°C and hermetically sealed. Temperature was measured with a Hydrolab thermistor or a YSI temperature-dissolved oxygen meter. Oxygen was determined by Winkler titrations (APHA 1989).

Settling particulate matter passing through the water column was collected by duplicate cylindrical sediments traps, 20 cm in diameter, with a 5:1 aspect ratio (Gardner 1980; Shafer 1988) and located 1 m from the sediment surface in Pallette, Russet, and Little Rock Lake. In Mirror Lake, traps were 7.4 cm in diameter (with a similar 5:1 aspect ratio) and four replicates were used. Trap material was collected at approximately 14 d intervals. Poisons or preservatives were not added to any of the traps.

Laboratory Analyses

Extraction

Filters and sediment trap material were extracted with 90% acetone. This solvent was chosen based on comparisons of extraction efficiencies (Bowles et al. 1985; Hurley 1988) and suggestions of other investigators concerning solvents that cause post-extraction pigment conversions (Mantoura and Llewellyn 1983; Jeffrey and Hallegraeff 1987). Samples were sonicated for 15 min, then stored for 24 h at 4°C and re-sonicated prior to filtering. Pigment extracts were filtered through 25-mm (diam.) Nylon 66, 0.2-μm filters (Alltech Assoc.). Ion paring agent (0.5 M tetrabutyl ammonium acetate in ammonium acetate buffer) was added to both samples and the initial solvent in the chromatographic gradient (see below) to aid in separation of polar derivatives.

HPLC Analysis

Pigment separations were performed using the following instrumentation: Waters Model 600 Solvent Delivery System; WISP 712A Autosampler with refrigeration unit (−4°C); Waters 481 Absorbance Detector (set at 440 nm); Waters 420C Fluorescence Detector (λ_{ex} = 420 nm; λ_{em} > 650 nm); and Waters Maxima chromatography software. Samples suspected of containing Bchl *a* were re-chromatographed and detected at 770 nm. Separations were performed on a 15-cm Waters NOVA-PAK C-18 column, protected by a C-18 guard column. Injection volumes ranged from 25 to 100 μL. The gradient, a modification of the system described by Mantoura and Llewellyn (1983), consisted of an initial solvent of 80% methanol, 20% 0.5 M tetrabutyl ammonium acetate in ammonium acetate with a 20-min linear gradient to 80% methanol, 20% acetone (Hooks et al. 1988). Final conditions were held for 25 min. Flow rate was held constant at 1.5 mL · min^{-1}.

Standards

Chl *a* and (Bchl *a*) were obtained from Sigma Co. Chl *a* derivatives were prepared by methods of Jeffrey (1968), Brown (1968), Barrett and Jeffrey (1971), and Sartory (1985) and specific absorption coefficients of Lorenzen and Downs (1986). Standards of lutein, zeaxanthin, β-carotene, fucoxanthin, and echinenone were generously donated by Hoffmann-LaRoche of Basle, Switzerland. Due to the lack of available commercial standards for other chlorophylls and carotenoids of interest (Bchl's *d* and *e*, myxoxanthophyll, oscillaxanthin, alloxanthin, diadinoxanthin, and peridinin), it was necessary to extract algal and bacterial cultures with known pigment compositions (c.f., Mantoura and Llewellyn 1983; Wright et al. 1991), collect peaks from column eluent, and re-dissolve in appropriate solvents with known pigment extinction coefficients. Extinction coefficients for non-commercially available standards were from Bchl *d* (Stanier and Smith 1960), Bchl *e* (Stal et al. 1984), and myxoxanthophyll, oscillaxanthin, alloxanthin, diadinoxanthin, and peridinin (Davies 1976). Confirmation of individual pigment peaks and identification constituents of over-lapping peaks was aided by in-line spectral analysis using a Waters Model 990 Diode Array Detector.

Results and Discussion

Water Column Pigment Distribution

Seasonal plankton dynamics were clearly evident from HPLC-derived pigment profiles in the four lakes studied. Both the depth of maximum pigment content and types and amount of phytoplankton and bacterial pigments differed among lakes. Pigment compositions reflected differences in major algal and phototrophic bacterial types among lakes.

In Little Rock Lake, large increases in pigment content were observed near the sediment surface (Fig. 1). In July, chl *a* levels were generally below 10 μg · L^{-1} in the upper 6 m of the water column; levels increased to about 15 μg · L^{-1} at 8 m and declined to <10 μg · L^{-1} at 9 m. However, at 9 m, Bchl *d* levels increased to concentrations >60 μg · L^{-1}, reflective of a peak in phototrophic green sulfur bacteria. Corresponding carotenoid profiles suggest that the deep water peak in chl *a* was due to both cryptophytes (alloxanthin) and green algae (lutein + zeaxanthin). Although lutein (green algae) is not effectively separated from zeaxanthin (blue-green algae) by our analyses, the lack of any other blue-green pigments (i.e., oscillaxanthin, myxoxanthophyll, or echinenone) support the hypothesis that the lutein + zeaxanthin peak was due to green algae.

In August, chl *a* levels from the surface to a depth of 8 m were below 5 μg · L^{-1} in Little Rock Lake. However, lower chl levels in surface waters and increased water clarity in the epilimnion, yielding greater light transmission to the hypolimnion, probably led to increased chl *a* levels at 8.5 m (>100 μg · L^{-1}). Similarly, Bchl *d* levels increased from about 60 to almost 200 μg · L^{-1} in this zone. It is interesting to note that, while the maximum chl *a* levels in July were associated with green algae and cryptophytes, the deep peak in August is due almost solely to cryptophytes. Oxygen levels at 8.5 m in July and August were below 1 mg · L^{-1} and the presence of cryptophytes in the low-oxygenated waters of Little Rock is consistent with observations in Lake Ciso reported by Pedros Alio et al. (1987).

In Russet Lake (Fig. 2), maximum pigment concentrations were found higher in the water column than those in Little Rock Lake. Russet Lake is a highly stained lake (high DOC, Table 1) and light penetration characteristics presumably are quite different from Little Rock Lake. Similar to Little Rock Lake, phytoplankton associated with deep chl *a* maximum varied during the summer. The relationship between chl *a* and Bchl *d* in this lake is interesting in that as chl *a* (and corresponding biomass) was compressed to a small region, Bchl *d* increased below the peak. This may be due both to decreased shading and to increased sulfide (for phototrophic bacterial growth) in the hypolimnion during stratification. In July, the deep carotenoid

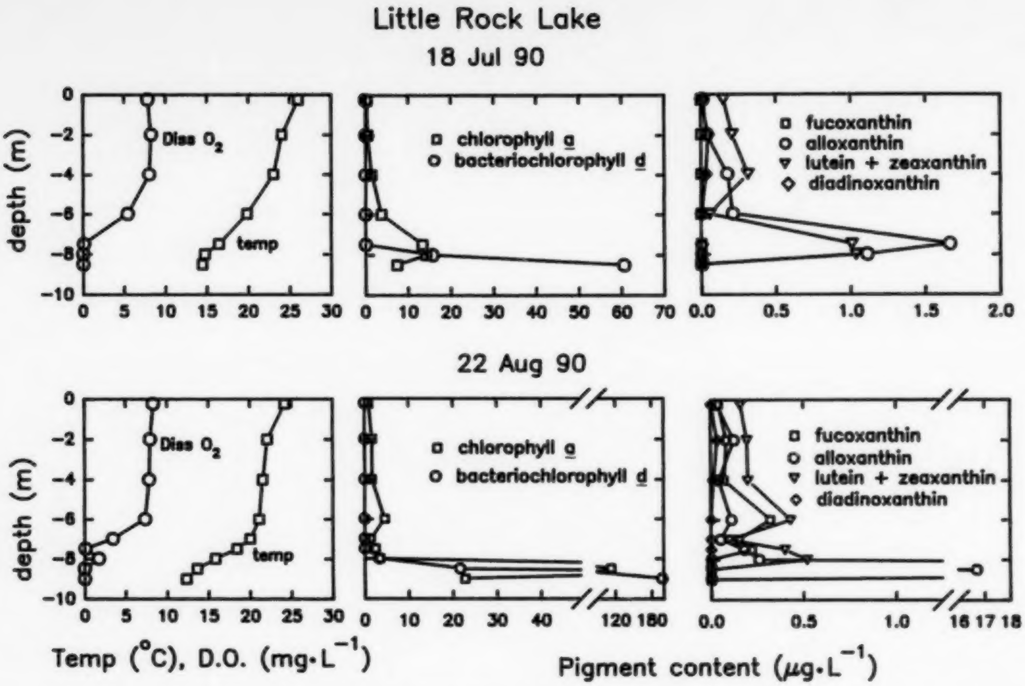

FIG. 1. Temperature, dissolved oxygen and pigment profiles for two selected dates in Little Rock Lake, Wisconsin.

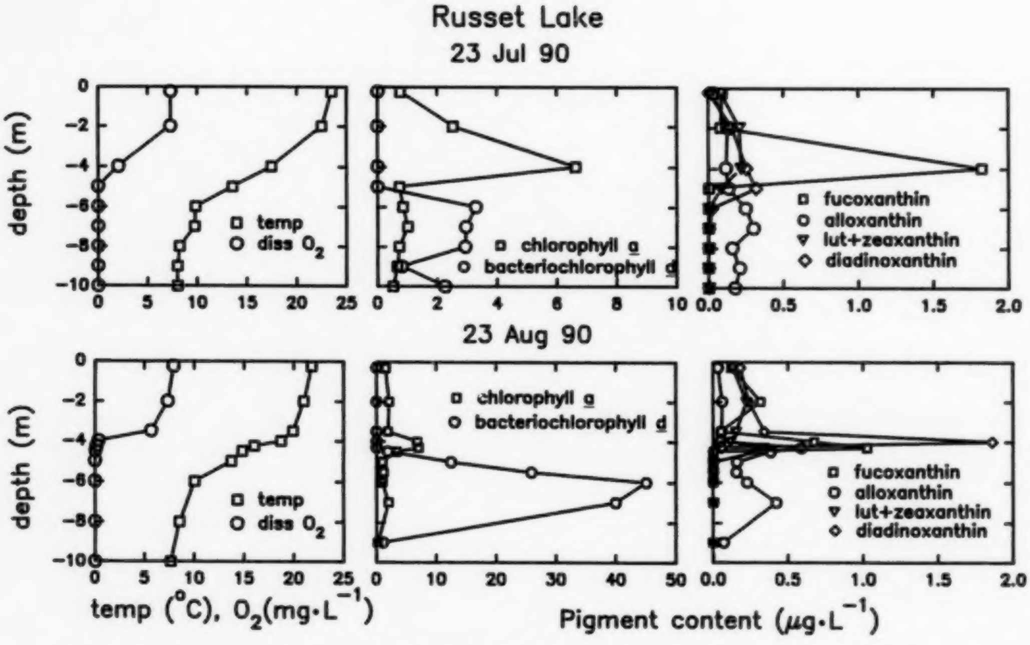

FIG. 2. Temperature dissolved oxygen and pigment profiles for two selected dates in Russet Lake, Wisconsin.

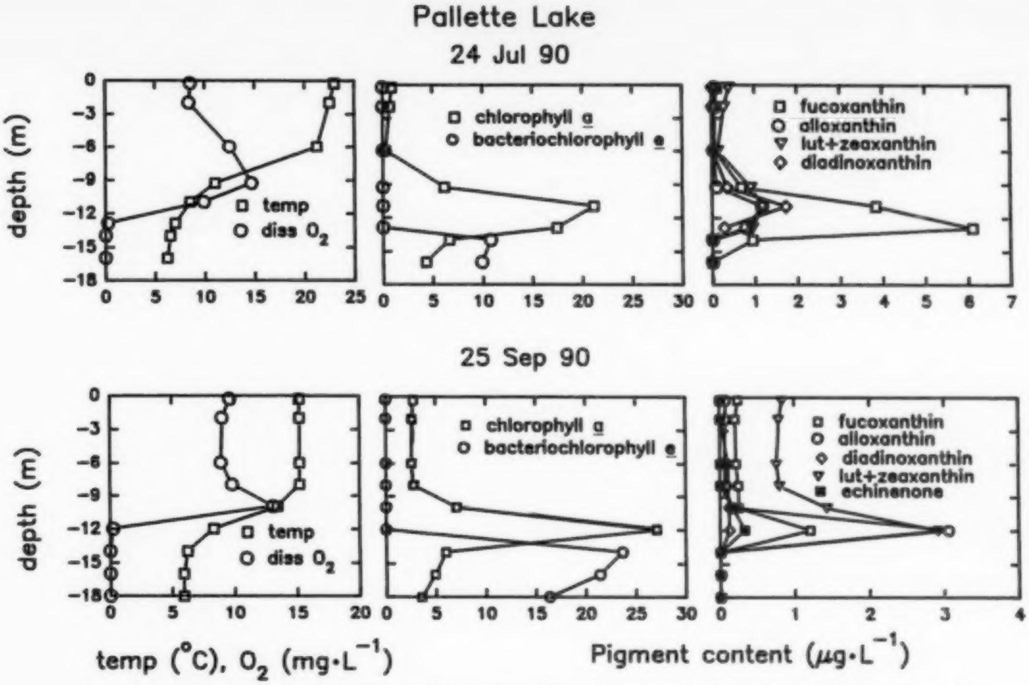

FIG. 3. Temperature dissolved oxygen and pigment profiles for two selected dates in Pallette Lake, Wisconsin.

peak was fucoxanthin, while in August the deep pigment peak was fucoxanthin, diadinoxanthin, alloxanthin, and lutein + zeaxanthin. Also similar to Little Rock Lake, cryptophytes appeared to be present in the hypolimnion at low oxygen levels. In Russet Lake, cryptophyte pigments were distributed over a wider region than in Little Rock. The absence of pheophytin further suggests that the cryptophytes present in the hypolimnion of Russet Lake were biologically active.

Pallette Lake (Fig. 3) is about 8 m deeper than Little Rock and Russet Lake. Following the spring bloom, chrysophytes were compressed into the metalimnion and accounted for the majority of the total carotenoid measured in July. As summer progressed, the deep phytoplankton peak was dominated more by cryptophytes and chlorophytes. In contrast to the other northern lakes, the only phototrophic bacteriochlorophyll was Bchl *e*, which was reflective of brown sulfur bacteria (see also Hurley and Watras 1991). Unlike Little Rock and Russet Lake, Bchl levels did not exceed 25 μg · L^{-1}.

In mesotrophic Mirror Lake (Fig. 4), pigment signatures of deep plankton were quite different from the three less productive northern Wisconsin lakes. While cryptophytes and chrysophytes dominated deep phytoplankton peaks in the northern lakes, deep production in Mirror Lake is due primarily to the blue-green alga *Oscillatoria agardhii* gomont (P.J. Garrison, unpublished data). The deep maximum of lutein + zeaxanthin in this lake was more reflective of zeaxanthin (blue-greens). This was further supported by similar increases in myxoxanthophyll and, to a lesser extent, echinenone. The relationship between light penetration and phototrophic bacterial production was also evident. As chl *a* levels in the deep maximum decreased from about 55 to about 30 μg · L^{-1}, Bchl *d* levels increased from about 70 to > 400 μg · L^{-1}. Similarly, the proportion of surface light at 6 m increased from about 4 to 8% (P.J. Garrison, unpublished data).

Effects of Deep Plankton on Water Quality Assessment

Clearly, nonuniformity of pigment profiles in lakes that possess elevated pigment concentrations at depth complicates the assessment of overall lake water quality, especially if water quality was based solely on surface water plankton or chlorophyll samples. In Pallette, Russet, and Little Rock Lakes, chrysophytes, cryptophytes, chlorophytes, and dinoflagellates dominated both the epilimnion and deep plankton zones. Therefore, surface water sampling may be qualitatively accurate of phytoplankton in the lake. Yet, concentrated zones of the algae in the hypolimnion would be overlooked in a quantitative estimate. Additionally, the presence of phototrophic bacteria would be overlooked. In mesotrophic Mirror Lake, nuisance blue-green algal blooms were absent in surface water, yet abundant in deep layers. Urbanization of the watershed and storm water inflow to Mirror Lake resulted in many nuisance blooms of blue-green algae in the 1970's and mitigative approaches were taken to eliminate these blooms from surface waters (Garrison and Knauer 1981). Although storm water diversion and alum treatment eliminated most problem blooms in the epilimnion, pigment analyses reveal that blue-green algae continued to persist but were concentrated deep in the water column.

Although the majority of plankton biomass was concentrated in a 3 m zone of the metalimnion and upper hypolimnion of Mirror Lake, estimation of whole lake pigment content (weighted by depth strata) was highly dependent on this zone. Surface water chl *a* levels were typical of whole-lake conditions

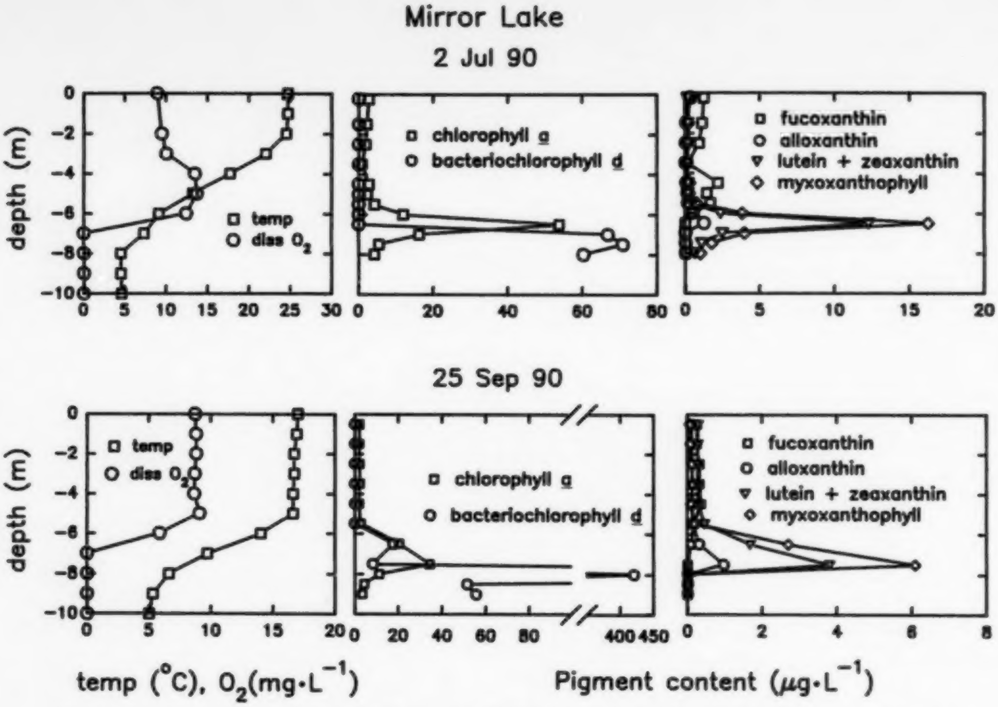

FIG. 4. Temperature, dissolved oxygen and pigment profiles for two selected dates in Mirror Lake, Wisconsin.

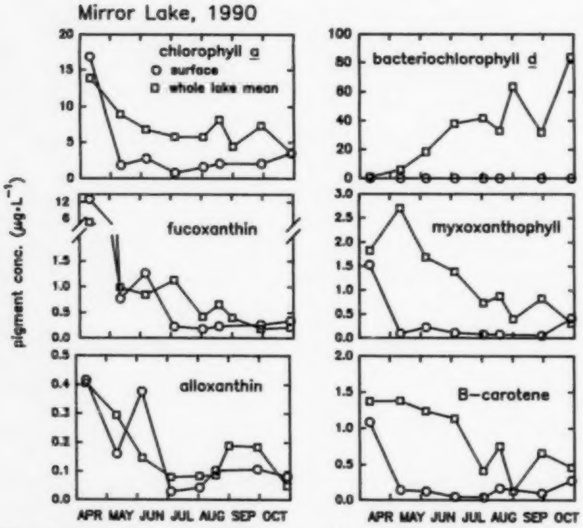

FIG. 5. Comparison of surface water and whole lake mean pigment content in Mirror Lake, Wisconsin.

only during overturn periods in April and October (Fig. 5). During most of the stratified period, chl *a* levels in surface waters were about a factor of five lower than whole-lake means. Bchl *d*, only found in anoxic waters, cannot be estimated from surface water samples. Interestingly, surface and mean whole lake fucoxanthin and alloxanthin levels are fairly well correlated during stratification. However, carotenoids associated with blue-green algae, myxoxanthophyll and β-carotene (β-carotene is present in most phytoplankton (Ricketts 1966; Johansen et al. 1974; Wright et al. 1991) but its relative abundance is much greater in blue-green algae (Hertzberg et al. 1971). This does not mean, however, that we assume that we can use β-carotene as an indicator of solely blue-green algae), clearly could not be estimated from surface samples.

Effects of Deep Plankton on Pigment Sedimentation

Paleolimnological applications of HPLC pigment analyses may involve reconstruction of phytoplankton histories based on carotenoid distributions in sediments (Leavitt et al. 1989; Hurley and Armstrong 1991; Hurley et al. 1992). Past trends in trophic control (i.e., zooplankton grazing) have been evaluated by quantifying chlorophyll degradation products (chl *a*, pheophorbide, and pheophytin) in sediment cores (Carpenter et al. 1986; Leavitt et al. 1989; Hurley et al. 1992). Interpretation of pigment stratigraphy is not straightforward, however. Typical HPLC pigment chromatograms can contain more than 100 peaks (Repeta and Gagosian 1987; Repeta 1989), complicating individual pigment separation. Additionally, the effects of differential diagenesis should be accounted for (Hurley et al. 1992). For example, peridinin was the dominant water column carotenoid in Lake Mendota, yet little was deposited in sediment traps and peridinin was not detected in bottom sediments. However, minor water column pigments, such as alloxanthin, diatoxanthin, and β-carotene were well-preserved in sediments (Hurley and Armstrong 1990).

Location of the bloom in the water column also may be an additional factor to consider when reconstructing a lake's planktonic history through pigment accumulation. In the case of

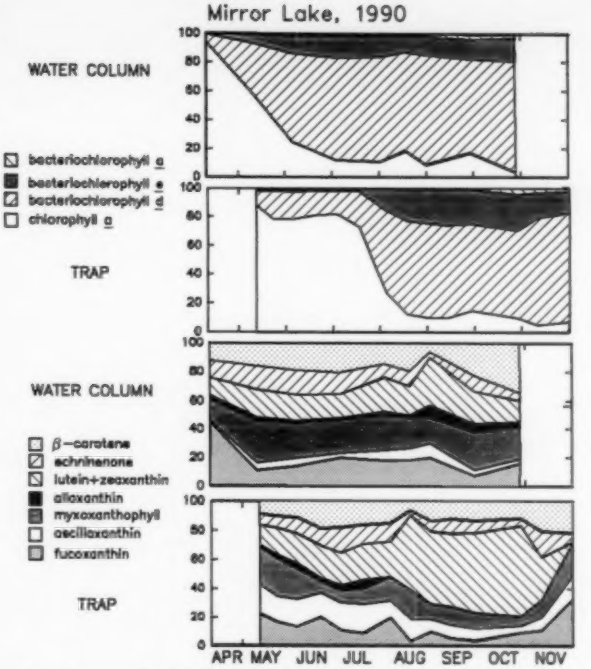

FIG. 6. Percent distribution of chlorophylls and carotenoids in the water column and sedimenting particulates in Mirror Lake, Wisconsin. Chlorophyll *a* represents the sum of chlorophyll *a* derivatives.

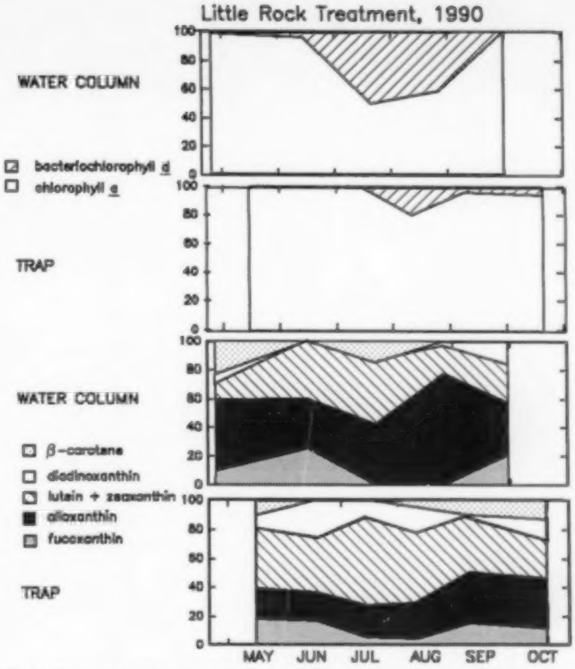

FIG. 7. Percent distribution of chlorophylls and carotenoids in the water column and sedimenting particulates in Little Rock Lake, Wisconsin. Chlorophyll *a* represents the sum of chlorophyll *a* derivatives.

Mirror Lake, the mean water column pigment concentration was clearly different from surface water concentrations. In contrast to surface blooms, senescent algae from deep blooms are subjected to degradation processes in the water column (cell lysis, photodegradation) for reduced time periods (Leavitt and Carpenter 1989; Leavitt and Carpenter 1990a). Therefore, deep blooms may be better preserved than surface blooms in bottom sediments. To test the influence of deep production on pigment sedimentation, we compared the mean composition of chlorophylls and carotenoids in the water column to that of sediment trap material.

Deep plankton clearly exerted a strong influence on pigment sedimentation in Mirror Lake (Fig. 6). After spring overturn, chl *a* dropped from 90% to less than 20% of the water column total chlorophyll (sum of chl *a* + Bchl's) for the entire summer. After about a two-month lag period, deposition of chlorophylls to sediment traps became dominated by bacteriochlorophyll. In addition to Bchl *d*, Bchl *e* (brown sulfur bacteria) and Bchl *a* (purple sulfur bacteria) also were important but at levels lower than Bchl *d*. Clearly, chlorophyllous pigments sedimenting from the water column did not reflect surface water pigment distribution. Similarly, blue-green algal pigments (oscillaxanthin, myxoxanthophyll, zeaxanthin, echinenone) dominated both the mean water column and settling pigment contents. If sediment trap material is indicative of material arriving at the sediment surface, and if these pigments were subsequently preserved in sediments, one might have interpreted these sedimentary pigment profiles as reflective of a eutrophic lake, despite the fact that surface water chl *a* levels rarely exceeded 5 μg · L^{-1}. In this lake, sedimenting carotenoid pigments (especially zeaxanthin, myxoxanthophyll, and oscillaxanthin) did not reflect surface water quality (commonly used to assess trophic status). Pigment paleolimnology–trophic status relationships used in other studies (Zullig 1989; Guilizzoni et al. 1982; Swain 1985) would thus be invalid for Mirror Lake.

Pigment deposition trends similarly reflected deep production composition in northern Wisconsin lakes. However, the types and amounts of pigments deposited at the sediment surface were quite different from those deposited in Mirror Lake. Blue-green algae were not important in deep production in these oligo-mesotrophic lakes and pigment composition (with the exception of Bchl) of the deep production was similar to surface waters. In Little Rock Lake, Bchl *d* reached levels near 200 μg · L^{-1} in the hypolimnion, while chl *a* levels in the epilimnion were 5 μg · L^{-1} (Fig. 1). Due to the relatively small volume of the hypolimnion, chl *a* remained the dominant water column chlorophyll. Similarly, Bchl *d* was relatively unimportant in trap material (Fig. 7). Comparison of water column to trap carotenoid composition revealed dominance of green algae and cryptophytes, indicated by the presence of lutein and alloxanthin, respectively. However, upon sedimentation, lutein was more significant than alloxanthin.

In contrast to Little Rock Lake, phytoplankton in Russet Lake were generally high in the water column, while Bchl *d* was distributed over the much deeper hypolimnion. In general, whole lake water column pigment trends were reflected in sediment trap pigment distribution (Fig. 8). Diadinoxanthin, a common pigment associated with dinoflagellates, comprised a smaller percentage of pigment in sediment trap material relative to water column content and may reflect differential diagenesis of carotenoid epoxides (Repeta and Gagosian 1987; Hurley and Armstrong 1990).

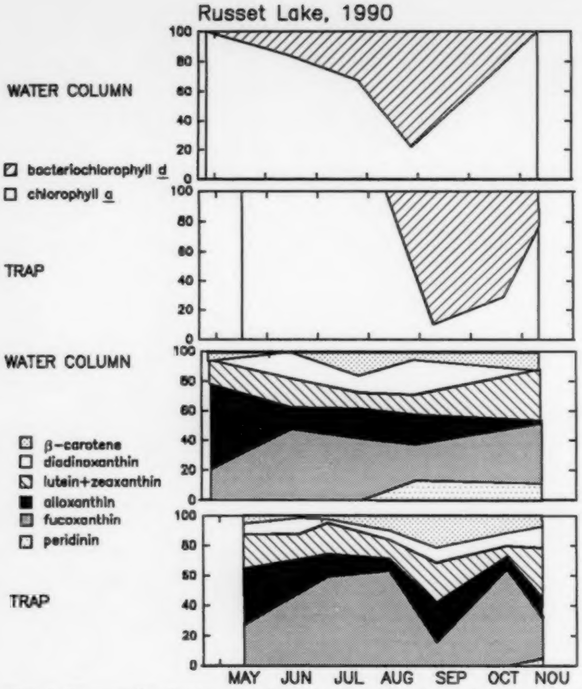

FIG. 8. Percent distribution of chlorophylls and carotenoids in the water column and sedimenting particulates in Russet Lake, Wisconsin. Chlorophyll *a* represents the sum of chlorophyll *a* derivatives.

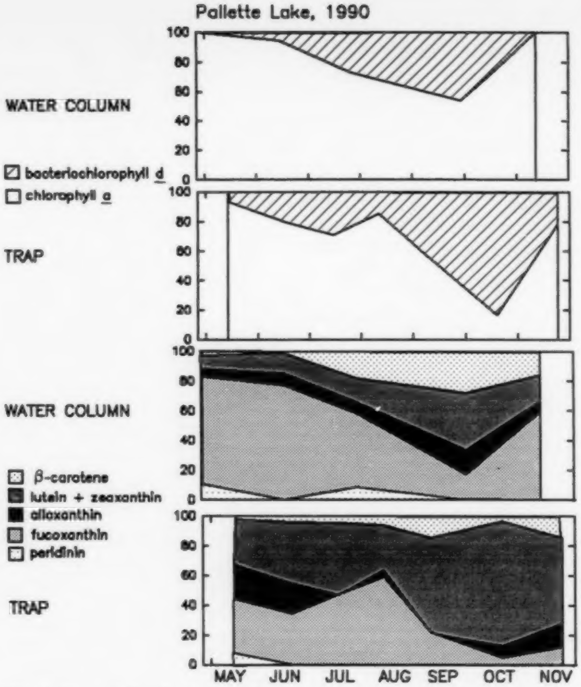

FIG. 9. Percent distribution of chlorophylls and carotenoids in the water column and sedimenting particulates in Pallette Lake, Wisconsin. Chlorophyll *a* represents the sum of chlorophyll *a* derivatives.

Pallette Lake, the deepest of the lakes studied, developed a fairly strong peak of brown sulfur (Bchl *e*) bacteria in the hypolimnion during summer (Fig. 9). During September, mean levels of Bchl *e* and chl *a* in the water column were similar, yet deposition to sediment traps, located near the deep Bchl peak, reflected bacterial dominance. Differential diagenesis during settling may have accounted for dissimilarities of the two chlorophylls. Dominance of the chrysophyte pigment fucoxanthin in the water column decreased upon sedimentation during May and June. By late summer and early fall, green algal pigments (lutein, and to a lesser extent, β-carotene) dominant in both the epilimnion and the deep peak, dominated pigment distribution in sediment trap material.

We recognize that sedimentation traps estimate a flux and that standing crop pigments do not reflect production of a given pigment. Therefore, appearance of a particular pigment as a sedimenting flux may be due to increased turnover rates. Additionally, the absence of a particular pigment in sedimenting material can be attributed to the lack of turnover and/or increased degradation upon settling. However, in all of the lakes studied, the influence of deep planktonic blooms is clearly important in the pigment flux that arrives at the sediment surface. Regardless of the individual production, preservation, or degradation process, deep planktonic communities exert a strong influence on pigment fluxes to surface sediments.

The common occurrence of deep plankton in these study lakes may resolve inconsistencies in estimating sedimentation and incorporation in other Wisconsin lakes (Hurley 1988; Hurley and Armstrong 1990; 1991). For example, in an anoxic basin of Trout Lake, Wisconsin, Hurley and Armstrong (1991) observed that alloxanthin accumulating in bottom sediments greatly exceeded deposition rates determined from sediment traps. Although sediment traps were placed at the base of the euphotic zone (1% light, at 17 m, a full 3 m above the sediments), this study suggests that if deep production in Trout Lake were similar to Little Rock Lake (Fig. 1), deep peaks of cryptophytes may have been present below the trap deployment depth. Since summer 1991 Trout Lake chlorophyll profiles revealed a deep Bchl maximum at 18 m (J.P. Hurley, unpublished data), it is reasonable to assume that alloxanthin may have been present and underestimated in the sedimenting flux.

Other studies have identified similar inconsistencies between water column and settling pigment distribution. For example, Leavitt and Carpenter (1990a) demonstrated that photooxidation and herbivore grazing controlled the types and amounts of pigments delivered from the photic zone to the sediment surface in three unproductive, north-temperate lakes. Similar to Hurley and Armstrong (1991), they noted an overabundance of alloxanthin settling to traps relative to the distribution of pigments in the photic zone (by a factor of between 2 and 5 during most of the summer in Peter and Paul Lake). It was concluded that high ingestion rates by zooplankton and subsequent deposition of rapidly sinking fecal material were responsible for the high rates of alloxanthin deposition to traps, a conclusion also supported by increased pheophorbide (a grazing indicator) in traps. Because these lakes are similar to other northern Wisconsin lakes in our study, it is reasonable to assume that deep cryptophyte peaks may have been present in anoxic (and aphotic) zones. Leavitt and Carpenter (1990b) also reported "unidentified chlorophyllous pigments" in sediment trap material with spectral characteristics similar to Bchl *d* (Hurley and Watras 1991) in Peter Lake; thus, a deep maximum of both phytoplankton (cryptophytes) and phototrophic bacterioplankton may have been present in this lake.

Conclusions

By applying HPLC techniques to identify pigment composition of four seepage lakes in north and central Wisconsin, we have shown that deep layers of mixed phytoplankton communities were a common occurrence. In lakes with sufficient hypolimnetic oxygen depletion, phototrophic bacterial communities can develop. The contribution of deep plankton pigment maxima to whole-lake values can be substantial.

The additional information gleaned from HPLC analyses of individual chlorophylls, bacteriochlorophylls, and carotenoids aids in determining the composition of planktonic communities throughout the water column and, moreover, enables the ability to rapidly estimate the composition of fine-scale planktonic layers. For instance, in the case of Mirror Lake over a 2-m interval in July, carotenoid levels increased by nearly an order of magnitude and chl *a* dominance was replaced by Bchl *d*.

Sedimentation of planktonic pigments also is substantially influenced by the presence of deep plankton. The signature of pigments deposited at the sediment surface may be quite different from that of pigments present in surface waters. Because deep production was common among the four lakes studied (typical for many Wisconsin lakes), the location of plankton blooms and their influence on pigment deposition must be considered in paleolimnological studies.

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Recruitment Distributions Revisited

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Fogarty, M.J. 1993. Recruitment distributions revisited. Can. J. Fish. Aquat. Sci. 50: 2723–2728.

Recruitment variability is a striking feature of the dynamics of many marine fish populations. Shelton (1992. Can J. Fish. Aquat. Sci. 49: 1754–1761) suggested that consideration of the survival probabilities of individuals or groups of individuals is the most appropriate strategy for modeling recruitment variability. A fundamental distinction can be made between environmental and demographic stochasticity. Here, environmental stochasticity refers to time-dependent variation in vital rates caused by environmental fluctuations. Demographic stochasticity is due to chance variation in the integer number of births and deaths in a population with time-invariant vital rates. The underlying basis for the simulations conducted by Shelton is consistent with the concept of demographic stochasticity. For large effective cohort size, these results converge to the deterministic case. Shelton's basic approach can be formalized using well-known results for a pure-death stochastic process. This derivation clarifies the importance of discrete processes for this class of models. Models of demographic stochasticity may be most useful for certain elasmobranch and marine mammal populations, while those incorporating environmental stochasticity may be more appropriate for many marine teleost and invertebrate populations. The critical distinction is between time-varying and time-invariant processes.

La variabilité du recrutement est une caractéristique remarquable de la dynamique de bon nombre de populations de poissons de mer. Shelton (1992. J. can. sci. halieut. aquat. 49 : 1754–1761) a avancé l'hypothèse à l'effet que l'examen des probabilités de survie de sujets distincts ou de groupes constitue la stratégie la mieux adaptée à la modélisation de la variabilité du recrutement. Il est possible de faire une distinction fondamentale entre l'aléatoire environnemental et l'aléatoire démographique. Dans ce cas-ci, l'aléatoire environnemental se rapporte à la variation en fonction du temps des indices vitaux attribuables à des fluctuations d'ordre environnemental. L'aléatoire démographique est attribuable à des variations au hasard du nombre entier de naissance et de décès dans une population dont l'indice vital ne varie pas en fonction du temps. La logique qui sous-tend les simulations de Shelton correspond au concept d'aléatoire démographique. Dans le cas de grandes cohortes effectives, ces résultats convergent vers le cas déterministe. La démarche fondamentale de Shelton peut être formalisée au moyen de résultats bien connus pour un processus aléatoire de mortalité simple. Cette dérivation précise l'importance de processus distincts pour cette classe de modèles. Les modèles aléatoires démographiques peuvent être les plus utiles dans le cas de certaines populations d'élasmobranches et de mammifères marins alors que les modèles à aléatoire environnemental peuvent convenir le mieux à deux nombreuses populations d'invertébrés et de téléostéens marins. La distinction critique est celle qui existe entre les processus qui varient avec le temps et ceux qui ne varient pas avec le temps.

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Shelton (1992) provided an interesting perspective on the expected form of recruitment distributions based on the probability of survival of individuals or groups of individuals. Shelton suggested that lognormal distributions, which have been proposed as appropriate recruitment distributions in several previous studies, cannot be supported based on theoretical considerations and that symmetrical recruitment distributions are to be expected under certain conditions. Here, it is shown that Shelton's arguments involve a fundamentally different form of stochasticity than those which lead to specification of lognormal-type recruitment distributions. A useful distinction can be made in this context between environmental and demographic stochasticity (May 1973, p. 30–36; Roughgarden 1979, p. 372–373). Environmental stochasticity refers to time-dependent variation in vital rates caused by environmental fluctuations. Demographic stochasticity is due to chance variation in the integer number of births and deaths in a population with time-invariant vital rates. My objective is to explore the assumptions underlying the two approaches, to review certain well-known results for both in an attempt to delineate the conditions under which each is most appropriate, and to relate these results to the simulations conducted by Shelton.

Models incorporating environmental stochasticity generally treat population size and time as continuous variables. In contrast, most models of demographic stochasticity consider population size and time as discrete variables. The latter class of models can be expected to be of primary importance for populations at relatively low population size (where the continuous approximation is not valid) and with negligible time-dependent variability in demographic rates. Models incorporating environmental stochasticity will often be more appropriate for the case of large population (cohort) size with temporal variation in vital rates. These approaches have different domains of validity and are appropriate under different circumstances.

Environmental Stochasticity

The development of recruitment models for populations with randomly varying mortality rates during the pre-recruit phase is reviewed below. For simplicity, the recruitment model is taken to be density independent but the general conclusions hold for more complex models (Fogarty, 1993). Here, the density-independent case is considered to be the appropriate null model and it is the form implicitly considered in Shelton's analysis. The mortality rate is assumed to vary as a result of fluctuations in environmental factors. Walters and Hilborn (1976) and Peterman (1978) provided lucid descriptions of the basis for invoking the central limit theorem in this general context. This intuitive argument was first used by MacArthur (1960) in an analysis of a density-independent population growth model. The principal elements of this approach are recapitulated below.

The rate of change of cohort size for the density-independent model is

$$(1) \quad \frac{dN(t)}{dt} = -\mu(t)\,N(t)$$

where $N(t)$ is the number in the cohort at time t and $\mu(t)$ is a time-varying instantaneous mortality rate. The solution is given by

$$(2) \quad \log_e N(t) = \log_e N_0 - \int_0^t \mu(\tau)\,d\tau$$

where $N_0 = N(0)$ is the initial number of viable eggs produced and t is the age at recruitment. The integral can be approximated by partitioning the prerecruit phase into an arbitrarily large number of intervals, giving

$$(3) \quad \log_e N(t) = \log_e N_0 + \sum_{i=1}^{n} \log_e s_i$$

where s_i is the survival rate during the ith interval:

$$(4) \quad s_i = \exp\left\{-\int_{m_{i-1}}^{m_i} \mu(\tau)\,d\tau\right\}$$

where m_i and m_{i-1} represent the upper and lower boundaries of the intervals. Notice that the s_i are random variables ($\mu(\tau)$ is a time-varying rate). The approximation is therefore readily interpreted as a sum of random variables. It is well known that the sum of independent, identically distributed random variables is approximately normally distributed for a sufficiently large number of observations according to the central limit theorem. Note that the number of intervals used to approximate the integral can easily be made sufficiently large to meet the convergence requirements under the central limit theorem.

It is perhaps less widely appreciated that the central limit theorem also holds under a much broader array of conditions than those specified above. The distributions need not be exactly identical (e.g., Feller 1957, p. 238–241; Parzen 1960, p. 430–433). Further, the assumption of independence can be relaxed for certain classes of stationary processes including finite Markov chains (Feller 1957, p. 374) and more generally for the case of finite dependence (Anderson 1971, p. 427). Feller (1957, p. 240) concluded that the central limit theorem in fact holds for "large classes of dependent variables."

Under the assumption of normally distributed mortality rates, the probability density function of recruitment for a given initial cohort size (N_0) and fixed t is lognormal:

$$(5) \quad P(N(t)\,|N_0) = \frac{N(t)^{-1}}{\sqrt{2\pi}\,\sigma_\mu t} \exp\left\{\frac{-[\log_e (N(t)/N_0) + \tilde{\mu} t]^2}{2\sigma_\mu^2 t^2}\right\}$$

where $\tilde{\mu}$ is the mean of $\mu(t)$ and σ_μ^2 is its variance. The mean recruitment for this model is given by

$$(6) \quad E(N(t)) = N_0 e^{-\tilde{\mu} t + (\sigma_\mu^2/2)t^2}$$

and the variance is

$$(7) \quad V(N(t)) = N_0^2 e^{-2\tilde{\mu} t + \sigma_\mu^2 t^2}\,[e^{\sigma_\mu^2 t^2} - 1]$$

(see Fogarty 1993 for a description and further specifications). The mean recruitment level is higher for the stochastic model than for the corresponding deterministic case by the factor $\exp[(\sigma_\mu^2/2)t^2]$. The coefficient of variation is given by

$$(8) \quad CV(N(t)) = [e^{\sigma_\mu^2 t^2} - 1]^{1/2}.$$

Notice that the coefficient of variation for the null model does not depend on the initial cohort size (N_0) and it increases monotonically as both t and the variance of the mortality rate increase.

These general conclusions are not dependent on the assumption of normality of mortality rates. Without specifying the probability distribution of mortality rates, it is possible to define the approximate mean, variance, and coefficient of variation of recruitment using the Delta method (see Fogarty 1993 for a description and assumptions in this context). Here it will be assumed that the variance in the mortality rate is relatively small so that higher order terms in the series expansion can be ignored. If we take the density-independent mortality rate $\mu(t)$ to be characterized by mean $\tilde{\mu}$ and variance σ_μ^2, the mean recruitment is

$$(9) \quad E(N(t)) \approx N_0 e^{-\tilde{\mu} t}\,\{1 + (\sigma_\mu^2/2)\,t^2]$$

to a first approximation and the variance is approximately

$$(10) \quad V(N(t)) \approx N_0^2 e^{-2\tilde{\mu} t}[\sigma_\mu^2 t^2]$$

(Fogarty 1993). The coefficient of variation is therefore

$$(11) \quad CV(N_t) \approx \frac{\sigma_\mu t}{\{1 + (\sigma_\mu^2/2)\,t^2\}}$$

to a first approximation. Notice that the mean is higher than the deterministic level by the factor $\{1 + (\sigma_\mu^2/2)t^2\}$ and that the coefficient of variation is independent of the number in the cohort as in the normal approximation case. These general features are distinctly different from those expected under demographic stochasticity (see below).

Demographic Stochasticity

Next, consider a model for the number of individuals in a cohort at time t based on time-invariant survival probabilities where the cohort size can assume only discrete values. The development below follows well-known results for a pure-death stochastic process and it is shown to underlie the basic model structure proposed by Shelton (see Bailey (1964, p. 90–91), Karlin and Taylor (1975, p. 158), and Pielou (1977, p. 13–14) for a full description of the method). In the following, it is assumed that the probabilities for each individual are independent and constant through time. The probability of an individual dying in an interval of time Δt is $\mu\Delta t + o(\Delta t)$ where $o(\Delta t)$ is a quantity of smaller order than Δt. The probability of obtaining exactly N individuals at time $t + \Delta t$ is given by

$$(12)\quad P_N(t+\Delta t) = P_{N+1}(t)\,\mu(N+1)\,\Delta t + P_N(t)\,(1-\mu N\Delta t)$$

where the first term on the right-hand side is the probability of exactly one death in a small interval of time Δt and the second term is the probability of no deaths in the interval. The interval is defined to be sufficiently small that the probability of two or more events is negligible. These probabilities are taken to be independent and therefore additive. The model can be expressed as

$$(13)\quad \frac{P_N(t+\Delta t) - P_N(t)}{\Delta t} = \mu\,(N+1)\,P_{N+1}(t) - \mu\,(N)P_N(t)$$

and taking the limit as $\Delta t \rightarrow 0$ and solving yields the probability density function (PDF) for recruitment. The PDF is binomial:

$$(14)\quad P(N(t)\,|N_0) = \binom{N_0}{N(t)}(e^{-\mu t})^{N(t)}\,(1-e^{-\mu t})^{N_0-N(t)}$$

with mean

$$(15)\quad E(N(t)) = N_0\,e^{-\mu t}$$

and variance

$$(16)\quad V(N(t)) = N_0\,e^{-\mu t}\,[1-e^{-\mu t}]\,.$$

The coefficient of variation is given by

$$(17)\quad \mathrm{CV}(N(t)) = \left\{\frac{1-e^{-\mu t}}{N_0\,e^{-\mu t}}\right\}^{1/2}.$$

The expression $\exp(-\mu t)$ is of course the probability of survival to time t and its complement is the probability of dying in this time interval. As noted by Shelton, an alternative derivation can be specified by considering this process as the outcome of N_0 independent Bernoulli trials where the probability of success is given by the exponential term above (see also Pielou 1977, p. 14). Note that there is no time variation in the mortality rate itself and therefore, $\exp(-\mu t)$ is a constant. Here, the variance emerges as a consequence of chance events in the integer number of individuals dying in small time intervals. The variance in recruitment is due to the fact that an individual can only be in one of two states at the end of the prerecruit period (alive or dead), while the expectation of survival will be a fraction between 0 and 1. Consideration of the sampling properties for such a process will quickly reveal that this binary designation is the source of the variance.

Note that in contrast with the model of environmental stochasticity, the mean is identical to the deterministic level and the coefficient of variation does depend on N_0; the coefficient of variation decreases with increasing N_0 and the results converge to the deterministic case. It is possible to construct discrete population models with time-varying parameters which incorporate elements of both approaches (see Appendix). Based on the theory of branching processes, Keiding (1975) demonstrated that under these conditions and at high population sizes, environmental stochasticity dominates. If the mortality rates are normally distributed, the recruitment distribution will again approach a lognormal distribution for large initial cohort sizes.

Comparison with Simulations

It next remains to place Shelton's simulations in this general context. Shelton considered two important modifications to the simple case described above: (1) groups or patches of eggs are defined and members of a group share a common fate (i.e., all individuals in the patch live or die) and (2) individuals or groups of individuals can have different survival probabilities. In the following, it will be most convenient to treat these cases as modifications to the basic Bernoulli trial derivation.

Consider first the case of no individual or group differences in survival probabilities. In Shelton's notations, $nE = N_0$ where n is the number of groups of eggs and E is the number of eggs per group, $p = \exp(-\mu t)$, and $q = (1 - \exp(-\mu t))$. Using Shelton's notation, the mean recruitment is then

$$(18)\quad E(N(t)) = nEp$$

and the variance is

$$(19)\quad V(N(t)) = nE^2pq\,.$$

The coefficient of variation is given by

$$(20)\quad \mathrm{CV}(N(t)) = \frac{\sqrt{npq}}{np}\,.$$

Note that the number of eggs per group (E) does not appear in the expression for the coefficient of variation. It will be helpful further to define the coefficient of skewness

$$(21)\quad G(N(t)) = \frac{(1-2p)}{\sqrt{npq}}$$

to permit comparisons with Shelton's simulations. The coefficient of skewness is also independent of the number of eggs per group.

For a sequence of life history stanzas each with an individual survival probability p_j, it is possible to take advantage of the above approach if the successive stages are independent as in Shelton's simulations. We then have

TABLE 1. Comparison of simulated (Shelton 1992) and exact results assuming bionomial process (in parentheses)[a]. The case designations correspond to Shelton (1992). The number of groups of eggs is designated n, E is the number of eggs per group, p_j is the survival rate in stage j, J is the number of stages, and CV is the coefficient of variation of recruitment (%).

Case	n	E	p_j	J	Mean	Variance	Skewness	CV
i	1×10^9	1	1×10^{-7}	1	100.26	98.49	–0.06	9.97
					(100.00)	(100.00)	(0.01)	(10.00)
ii	4×10^7	25	1×10^{-7}	1	100.48	2539.03	0.53	50.15
					(100.00)	(2500.00)	(0.50)	(50.00)
iii	1×10^4	1	0.5	5	312.27	309.51	0.01	5.63
					(312.50)	(302.73)	(0.003)	(5.56)
iv	1×10^2	100	0.5	5	318.78	31551.62	0.62	55.72
					(312.50)	(30273.43)	(0.54)	(55.68)
v	1×10^4	1	0.75	10	562.72	521.54	0.03	4.05
					(563.14)	(531.42)	(0.04)	(4.09)
vi	1×10^2	100	0.75	10	567.76	53883.36	0.38	40.88
					(563.14)	(53142.30)	(0.38)	(40.93)

[a]The results are exact for cases i and ii and approximate for cases iii–vi (exact results for the latter are possible but would require the exact sequence of random numbers chosen for the survival probabilities in the simulations). The variance is overestimated in the approximation. The simulations can also be effectively represented using a Poisson approximation to the underlying binomial model (G. Mertz and R.A. Myers, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, NF A1C 5X1, Canada, personal communication).

$$(22) \quad p = \prod_{j=1}^{J} p_j$$

and $q = 1 - \Pi p_j$. For the case of identical p_j, as in Shelton's simulations, $p = p_j^J$ where J is the number of life history stages. It should be noted that the specification of the J life history stages in this context again involves time-invariant rates for each stage and does not correspond to the arguments invoked in the case of environmental stochasticity (see Equations 3 and 4). Rather, this approach should be viewed as the development of a multistage model with no time variation in death rates within stages.

For the case where individual differences in survival probabilities occur (Shelton's cases iii–vi), further modifications are necessary. Feller (1957, p. 216–217) provided an overview of Bernoulli trials with different probabilities among trials (in this case, individuals).

The mean for Shelton's simulations is now

$$(23) \quad E(R) = E \sum_{i=1}^{n} p_i$$

and the variance is given by

$$(24) \quad V(R) = E^2 \sum_{i=1}^{n} p_i q_i .$$

Interestingly, the variance is lower for the case where the survival probabilities vary among individuals relative to the case of no interindividual variation (see Feller 1957, p. 217). The coefficient of variation is then

$$(25) \quad \mathrm{CV}(R) = \frac{\left\{ \sum_{i=1}^{n} p_i q_i \right\}^{1/2}}{\sum_{i=1}^{n} p_i} .$$

Extension to the case of multiple stages follows directly from the previous arguments.

A comparison using the analytical results in the preceding sections and Shelton's simulations is provided in Table 1. Analysis of the case of interindividual differences in survival probabilities (cases iii–vi) would require the exact sequence of random numbers chosen for the survival probabilities in the simulations. Here, this process is approximated by setting the survival probabilities to the mean of the uniform distributions in Shelton's simulations and using Equations 19–23. The exact results under the model of demographic stochasticity (cases i and ii) closely correspond to the simulation studies (Table 1). Further, the approximate results for cases iii–vi also are in close agreement with the simulation results, indicating that the particular form of interindividual differences examined by Shelton does not markedly affect the outcome.

Shelton reported that for the case of independent survival rates of each individual in the cohort, the recruitment distributions are nearly symmetrical (Shelton's cases i, iii, and v). For the case of "dependent" survival (i.e., all members of a group or patch either survive or die), asymmetrical recruitment distributions are obtained in the simulations (cases ii, iv, and vi). The positive skewness for these cases is due entirely to the lower value of n in the dependent survival case relative to the corresponding independent survival case as clearly noted by Shelton. Because the number of individuals in a group (E) does not appear in the expression for the coefficient of skewness, the issue of complete dependence of survival within a group does not directly affect the outcome. It does of course indirectly affect the outcome by reducing the "effective" number of groups (n) if the total number of eggs (nE) is held constant as in the simulations (i.e., n necessarily decreases as E increases for constant nE). The constraint of constant nE in the simulations is reasonable based on energetic grounds. It is useful to consider the case of no constraints, however, to illustrate the relative roles of the various terms in the model.

The two types of time-invariant processes considered by Shelton (independent and complete within-group dependence) represent extremes in a continuum of possible outcomes. It appears unlikely that the survival probability of individuals during prerecruit stages is entirely independent for the case where $E = 1$. The distribution patterns of the early life stages of many species are patchy and it is probable that the fate of individuals within a patch is related. For example, advective processes resulting in loss of some or all individuals in a patch, encounters between a predator field and the patch, etc., would tend to result in a dependence in survival rates within a patch. This dependence, however, need not be complete as in the second class of processes described by Shelton. It is possible to consider the case of dependent Bernoulli trials (e.g., see Parzen 1960, p. 113–134) to deal with this case. Shelton's approach has the merit of focusing attention on the issue of patch dynamics and its implications for recruitment processes for populations with negligible time-dependent variation in mortality rates.

Conclusions

The modeling strategy chosen to represent variability in recruitment processes depends fundamentally on issues such as the effective number of individuals in the cohort (i.e., whether the large sample approximation is valid) and whether demographic rates and processes vary with time. Many marine populations, notably teleosts and invertebrates, are characterized by high fecundity, small progeny size, and broadcast spawning strategies. These life history characteristics virtually assure that mortality rates will vary during the prerecruit phase (Fogarty et al. 1991). Models incorporating environmental stochasticity are therefore required to represent fully the dynamics of these populations. Even relatively small coefficients of variation in mortality rates result in large recruitment fluctuations in these populations (Fogarty 1993).

The intuitive derivation invoking normality of mortality rates under the central limit theorem is robust under a broad range of conditions. However, as noted by Shelton, the application of any modeling approach should be made on a case by case basis. Clearly, the assumption of normality of mortality rates would be inappropriate for the case of nonstationarity or other conditions leading to a slowly decaying autocorrelation function in the time-varying case. The advantage of invoking the normal approximation in this context is its analytical tractability (see also Shelton 1992). Dennis and Patil (1991) provided a comprehensive review of applications of the lognormal distribution in ecology and suggested that it should be viewed as a convenient analytical approximation to more complicated processes. It should be stressed that the concept of environmental stochasticity is, however, not contingent on the assumption of normality of demographic rates (see Equations 9–11) and alternative approaches can be specified if it is inappropriate to invoke the central limit theorem.

Models of demographic stochasticity converge to the deterministic case for large population or cohort size. This result is clearly evident in Shelton's simulations where the coefficients of variation for the case of individual survival probabilities ($E = 1$) are ≤10%. Observed coefficients of recruitment for marine teleosts are typically on the order of 100% and it is unlikely that measurement error alone can account for this discrepancy.

Empirical studies of survival rates, life history characteristics, and recruitment distributions (e.g., Allen 1973; Hennemuth et al. 1980; Peterman 1981; Garrod 1983; Myers et al. 1990) are invaluable in understanding broad patterns in recruitment dynamics and in providing the basis for comparative studies across taxonomic groups. Shelton correctly noted that measurement error must be considered in any such evaluation of actual observations. However, the issue of measurement error should not obscure the fundamental question of whether the demographic rates are subject to temporal variation. If mortality rates do vary in time during the prerecruit phase, demographic stochasticity alone cannot account for the expected form of recruitment fluctuations. It is of course possible to construct discrete population models with time-varying parameters which might be more appropriate for small effective sizes (see Appendix).

General life history characteristics, including issues such as bet-hedging (Shelton 1992), clearly deserve further attention in the study of recruitment processes. For populations with no time-dependent variation in mortality rates, Shelton's simulations demonstrate that independence in survival among individuals increases the effective cohort size, resulting in lower variance in recruitment and symmetrical recruitment distributions. For populations subject to environmentally driven variability, different life history characteristics can be expected to affect the variance in mortality and the shape and dispersion of the recruitment distribution. Factors resulting in a lower variance in mortality will of course result in lower recruitment variability.

Consideration of individual differences in survival probabilities adds an important dimension to recruitment studies. Shelton did examine the implications of survival differences among individuals or groups of individuals. For the particular simulation structure considered by Shelton, variation in survival among individuals or groups did not result in large-scale departure from the results expected when the mean survival rate is substituted for the individual survival probabilities (see Table 1). Development of individual-based recruitment models does, however, permit consideration of issues that cannot be addressed in the context of more traditional recruitment models and further work on this topic should be encouraged. For a more general discussion of individual-based modeling approaches, see Huston et al. (1988). Caswell and John (1992) noted that in many cases, individual variation can be effectively represented by specifying a probability distribution for demographic parameters if certain mixing assumptions are met and tracking individuals is not necessary. However, if individuals interact locally, it will be necessary to track individuals by simulation.

It is important to note that the variation in individual or group survival probabilites considered by Shelton (cases iii–vi) addresses a different issue than the time-varying survival rates considered in the models of environmental stochasticity. It is, however, possible to construct models that consider time-varying survival rates which differ among individuals or groups and therefore contain elements of both approaches.

Shelton's study highlights an important class of stochastic processes that has not been given sufficient attention in previous studies of recruitment variability. These issues can be expected to be particularly important for populations that are relatively insensitive to environmental variation and that have been driven to low effective population sizes (e.g., marine mammals and certain large sharks). These species produce large, well formed progeny which are less vulnerable to exogenous variability than most marine teleosts and invertebrates. Shelton's approach provides important insights into the sources of variability in populations of this type. For species characterized by time-varying mortality rates, lognormal-type distributions will often provide reasonable recruitment distributions as a first approximation to portray the main features of the expected recruitment fluctuations. Careful consideration of life history characteristics and sources of variability, including exogenous or environmentally induced stochasticity and individual variability due to genetic and other factors, should be made to inform the modeling strategies used to represent the recruitment dynamics of marine populations.

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Appendix

The case of time-invarying vital rates can be readily accommodated in the pure-death stochastic model. If the instantaneous mortality rate does vary with time, the PDF is then

$$P(N(t) \mid N_0) = \binom{N_0}{N(t)} \left\{ \exp\,[-\int_0^t \mu\, d\tau] \right\}^{N(t)} \times \left\{ 1 - \exp\left[-\int_0^t \mu\, d\tau\right] \right\}^{N_0 - N(t)}$$

with mean

$$E(N(t)) = N_0 \exp\,[-\int_0^t \mu\, d\tau]$$

and variance

$$V(N(t)) = N_0 \left\{ \exp\left[-\int_0^t \mu\, d\tau\right] \right\} \left\{ 1 - \exp\left[-\int_0^t \mu\, d\tau\right] \right\}.$$

The coefficient of variation is given by

$$\mathrm{CV}(N(t)) = \left\{ \frac{1 - \exp\,[-\int_0^t \mu\, t]}{N_0 \exp\,[-\int_0^t \mu\, t]} \right\}^{\frac{1}{2}} .$$

Without specifying the form of the time dependence, it is not possible to further define the model. However, it is possible to again invoke the central limit theorem in approximating the integral. The distribution in that case of course would no longer be binomial and for large effective cohort sizes, the environmental variability would dominate.

PERSPECTIVES

On Recruitment and Distribution of Atlantic Cod (*Gadus morhua*) off Newfoundland

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deYoung, B., and G.A. Rose. 1993. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland. Can. J. Fish. Aquat. Sci. 50: 2729–2741.

The Atlantic cod (*Gadus morhua*) populations located off Labrador and Northeastern Newfoundland (NAFO areas 2G–3L) have recently declined to the lowest levels of abundance on record. These "northern" cod have historically comprised several geographically recognizable populations with independent migratory life cycles on the shelf from the Grand Banks to Labrador. A reappraisal of past and recent work suggests that fundamental changes have taken place in the population dynamics of these cod during the past several decades. We focus on two key elements: distribution and recruitment. Distributions have become more southerly and recruitment failures prevail. We argue that these features are related and that northerly spawning and warm ocean conditions are prerequisites for strong recruitment. Cold ocean temperatures are associated with southerly distributions and poor recruitment. We propose the "right site" hypothesis, that egg and larval retention and survival are spatially dependent and that in cold years, spawning tends to occur at southerly locations where larval retention will be poor. We make several testable predictions: regeneration of the northern populations will occur slowly at time scales of decades, regeneration of southern populations will occur more quickly given warming conditions, and the 1991–93 year classes will be poor because of southerly distributions.

Ces dernières années, les populations de morues franches (*Gadus morhua*) au large du Labrador et dans le secteur situé au nord-est de Terre-Neuve (divisions 2G–3L de l'OPANO) sont tombées à leurs plus bas niveaux jamais enregistrés. Historiquement, ces morues « du Nord » regroupement plusieurs populations géographiquement distinctes qui ont des cycles migratoires indépendants sur le plateau continental, des Grands Bancs jusqu'au Labrador. Un réexamen des articles récents et antérieurs donne à penser qu'il y a eu des changements fondamentaux dans la dynamique démographique de ces morues au cours des dernières décennies. Prenons deux éléments essentiels : la distribution et le recrutement. Les morues sont maintenant distribuées plus au sud et le recrutement fait défaut. Nous avançons l'hypothèse à l'effet que ces caractéristiques sont reliées, et que le frai dans des eaux nordiques et qu'une température élevée de l'eau sont des prérequis d'un bon recrutement. La froideur de l'eau est associée à des distributions méridionales et à de mauvais recrutements. Nous avançons l'hypothèse « du bon endroit », où la rétention et la survie des oeufs et des larves ont des contraintes spatiales et que, les années, où l'eau est froide, le frai tend à se faire plus au sud, où la rétention des larves est mauvaise. Nous formulons plusieurs prédictions vérifiables : la régénération des populations boréales se fera lentement, en termes de décennies, la régénération des populations méridionales se fera plus rapidement si l'eau se réchauffe, et les classes d'âge 1991–1993 seront peu abondantes à cause de la distribution plus méridionale.

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Atlantic cod (*Gadus morhua*) historically have occupied the coastal shelf from northern Labrador to the southern Grand Banks (Fig. 1A). The cod from northern Labrador to the northern Grand Banks have been termed the "northern" cod stock (NAFO areas 2G–3L) (Fig. 1A), traditionally the most productive cod stock in Atlantic Canada (Rose 1992). In recent times the abundance of northern cod has declined to the lowest levels on record (CAFSAC 1992). Cod have become commercially extinct in the most northerly portion of their former range off Labrador (2GHJ). Under the existing population dynamics paradigm for the stock, the probable causes of this decline are several, including overfishing by local and foreign vessels, recruitment failures as a result of low numbers of spawning fish and poor environmental conditions, and possible increased mortality caused by a growing seal herd and other factors. The prevailing interpretation (e.g., Harris 1990) suggests that removal of these factors will result in a natural rebuilding and redistribution of the stock to levels and patterns last seen some 30 yr ago (Fig. 1B). In this perspective, we question this interpretation and several of its assumptions. We

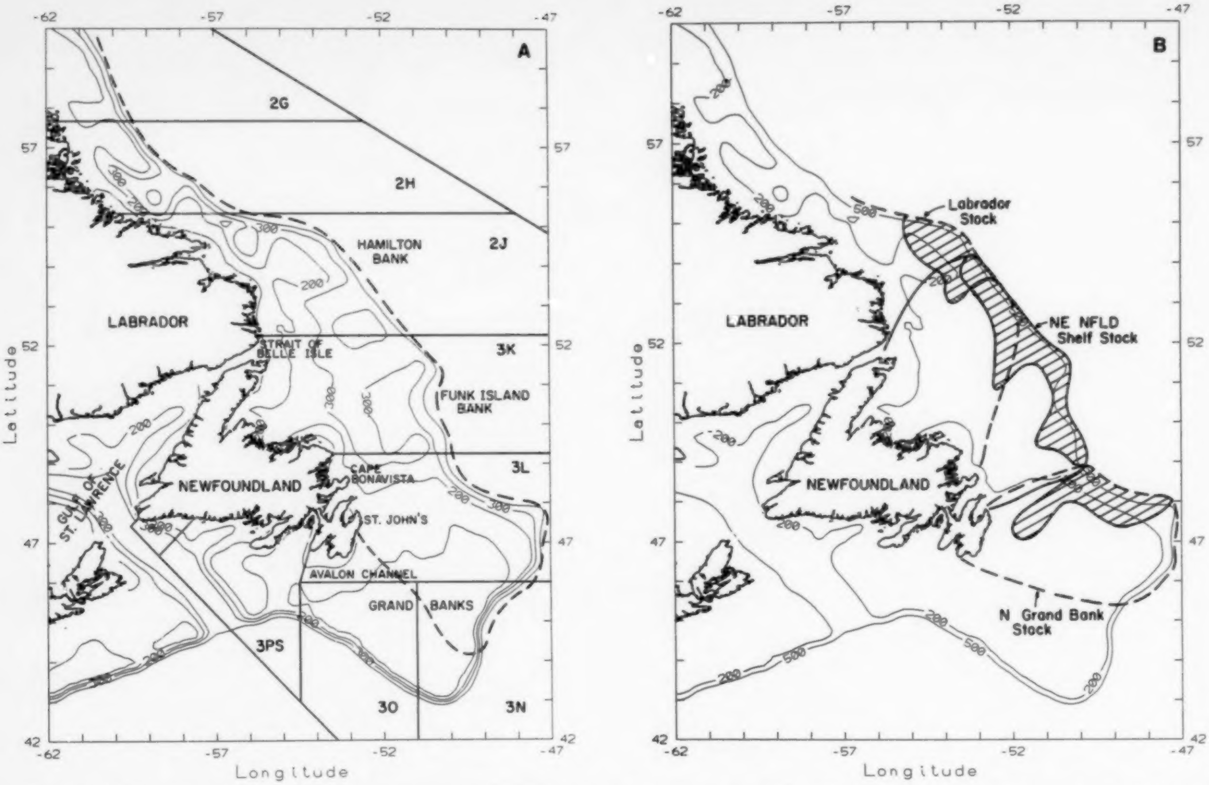

FIG. 1. (A) Bottom topography of the area encompassing NAFO statistical divisions from 2G to 3Ps. Historical range of the northern cod "stock complex" taken from Templeman (1966) is indicated by the broken line. (B) Historic distributions of the Labrador, northeastern Newfoundland, and northern Grand Banks populations. Spawning regions of the different stocks are hatched.

reassess the interactions between stock spatial dynamics and distributions, recruitment, abundance, and environmental fluctuations on the Newfoundland and Labrador shelves and develop an alternative model of recent stock fluctuations.

Historical Stock Distribution

A fundamental component of fisheries population models and management programs is the distribution of the stock in space and time (Hilborn and Walters 1992). Here, a stock is a management unit whereas a population is a self-sustaining group of organisms whose dynamics, especially recruitment and mortality, are for the most part independent of other populations. A stock may include single or several populations. This definition does not invoke genetic isolation as a prerequisite for population status (see Gauldie 1991).

Population dynamics models employed in research and management of the northern cod have traditionally treated these fish as a single stock (CAFSAC 1992), although there has been wide recognition that the northern cod might form a "stock complex" composed of several populations (Halliday and Pinhorn 1990). In keeping with the single stock notion, recruitment and mortality have often been considered to have no important spatial properties within the full stock area (e.g., Helbig et al. 1992). The Northern Cod Review Panel (Harris 1990) provided a detailed review of the "northern" cod stock as part of an independent review of the management strategy of the Department of Fisheries and Oceans and described the northern cod as a "stock complex". Harris (1990) argued that all regions from northern Labrador (2G) to the southern Grand Banks (3NO) could be considered as one management unit but also recognized that this was an important issue requiring further research.

Several lines of evidence suggest that the "northern" cod has historically included several populations whose summer distributions often overlap because of large-scale shoreward and along shore searches for food. The most longstanding evidence for this view has been derived from 60 yr of cod tagging studies in Newfoundland and Labrador waters and in the Gulf of St. Lawrence. Tagging studies have consistently suggested that local races exist within the range of the "northern" cod.

Thompson (1943) summarized the earliest studies: "On the whole, the evidence from tagging experiments supports the general hypothesis of the existence of local races of cod, the bulk of each of which, in early years of life at least, remain within a region where a specific range of hydrographic and feeding conditions prevails." This hypothesis was supported by more extensive tagging conducted between 1962 and 1966 (May 1966; Templeman 1979). May (1966) suggested that three major groups existed: (1) a Labrador stock that spawns on Hamilton Bank and feeds along the coasts of Labrador and northern Newfoundland, (2) a northeastern Newfoundland population that also spawns on Hamilton Bank but also southward to Funk Island Bank and feeds in summer along the coast of northeastern Newfoundland, and (3) an eastern Newfoundland population that spawns on the northern Grand Bank and feeds in summer along the coast of eastern Newfoundland. Extensive offshore tagging studies conducted from 1978 to 1981 (Lear 1984) supported May's (1966) hypothesis and provided much additional evidence that the northeastern Newfoundland stock may occupy a large range extending over the entire Funk Island Bank region and as far north as the Hamilton Bank; these data also showed that post-spawning movements tend to be southward from more northerly spawning sites. Tagging studies (1990–92) designed to track the seasonal and annual movements of cod on the northeastern Newfoundland shelf support the earlier work and further indicate that migratory circuits are to a large extent spatially repeated from year to year (Rose 1993).

Northern cod have also been reported to exhibit differing biological properties from the north to the south of their overall range. Thompson (1943) and Templeman (1981) reported that vertebral counts differed between Labrador and Grand Banks fish. Templeman and Fleming (1963) showed that parasitic burdens could be used to characterize cod found in these same zones. These differences suggest limited north–south mixing and therefore geographic stability at scales smaller than those of the full northern cod range. Whether these differences are genetically based remains uncertain. Genetic methods have been suggested for differentiating fish populations, but there is controversy about their validity (Gauldie 1991, 1992; Bentzen 1992). The Ocean Production Enhancement Network (OPEN) and the Northern Cod Science Program (NCSP) are investigating whether there is genetic distinctness among the geographically disparate cod populations from Labrador to the Scotian shelf.

The composite evidence from tagging, biological descriptors, and the work and views of Thompson (1943), May (1966), Templeman (1979), Lear (1984), and Halliday and Pinhorn (1990) suggests that the "northern" cod stock is composed of three geographically based populations (Iles and Sinclair 1982) associated with the Labrador Banks, the northeastern Newfoundland shelf, and the northern Grand Bank (Fig. 1B). All three populations typify North Atlantic cod in that they are migratory and have supported large fisheries. To quote Thompson (1943), "sedentary cod (called "natives" by fishermen) are found all along the coast, but do not as a rule support a large fishery. As a rule, in all regions of the distribution of cod, the fish of the *extended* migration type support the large fisheries." A summary description of each follows.

Labrador

The Labrador cod spawn on the Labrador shelf (Fig. 1B) and migrate in summer to the Labrador coast, at times moving into the Strait of Belle Isle and northern Gulf of St. Lawrence and also to northeastern portions of Newfoundland. The Labrador population almost certainly supplies recruits to more southerly fisheries on the northeastern Newfoundland coast. We propose that recruits are also supplied to the southerly spawning populations on the northeastern Newfoundland shelf and northern Grand Banks. We believe that the Labrador population is the most important to the fisheries because its more northerly distribution provides the best location from which to generate high levels of recruitment. It may also be in the most vulnerable location to changing ocean conditions. The Labrador fish are accustomed to cold waters (Thompson 1943) and are slow growing with high vertebral numbers (Templeman 1979). This stock was decimated by European trawlers in the 1960's (Harris 1990), though it did partially rebuild during the late 1970's and early 1980's. At present, the population is at the lowest level on record. In the northern reaches (2GH), cod have been commercially extinct since the 1960's.

Newfoundland Shelf

The northeastern Newfoundland cod are the most migratory and widest ranging population. They may spawn from Hamilton Bank to the northernmost parts of the Grand Bank depending on their abundance and environmental conditions. This population may recruit well only when spawning occurs in the northern reaches of its range. Recruitment may be as strong as that from the Labrador population when spawning occurs in the northerly part of its range. Fish of this population are midway in growth and meristic characteristics between the Labrador and Grand Banks populations. The northeastern Newfoundland cod migrate in summer to coastal northeastern Newfoundland from the Avalon northward to the Strait of Belle Isle.

Northern Grand Bank

The northern Grand Bank population spawns along the northern and eastern slopes of the Grand Bank and migrates in summer along the northeastern slopes of the Bank to the Virgin Rocks area and shoreward to the Avalon Peninsula, and at times northwards to Cape Bonavista. Fish of this population are faster growing and have lower vertebral numbers than fish of the more northerly populations (Templeman 1979). Northern Grand Bank cod are now at relatively low levels.

The historic model of the stock structure of cod on the northeastern Newfoundland and Labrador shelves generates many questions about how such a population pattern could be maintained. The model assumes that widely dispersed progeny congregate with their mother population before they mature. This is not a trivial assumption. Indeed, how can a young cod that was spawned far to the north, drifted southward as an egg, and then spent its entire postdrift life away from its natal area retrace a voyage it made only once as an egg? Templeman (1979) invoked "homing" as the answer. But homing typically assumes a period of juvenile residence in the natal area so that imprinting can occur, as in salmon (Quinn and Leggett 1987). Cod could be genetically predisposed and equipped to return to spawn with their mother population at their natal areas (or at least within broad but distinct regions). However, neither homing nor any navigation mechanisms capable of sustaining such a return migration have been demonstrated for this species. The tagging data suggest that once adult migration patterns are established, they are sustained, but these data are not compelling evidence for homing to natal areas. Juveniles could learn migration routes by following adults back to the spawning regions. But how could juveniles recognize adults of their population, since adults of different populations have overlapping distributions at various times during their life history?

A related but simpler model of the stock structure suggests that populations are sustained by juvenile fish resident in the area of the adult population joining that population. Although the returning juveniles are likely to be progeny of the adult population, at times this may not be the case. Hence, populations are geographically rather than genetically based (although in some cases, genetic differences may have evolved, but their

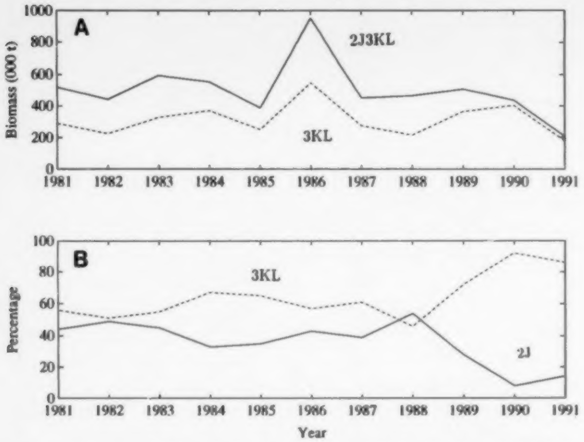

FIG. 2. (A) Trawlable biomass for total "northern" cod stock (NAFO divisions 2J3KL) (solid line) and for the southern region (3KL) (broken line). (B) Percentage biomass in the northern region (2J) (solid line) and in the southern region (3KL) (broken line). Data are from Baird et al. (1992).

importance is unclear). In this model, juvenile cod residing along the Newfoundland and Labrador coast move progressively seaward and closer to the shelf spawning areas as they mature and then join the adjacent adult population. Hence, stock structure will depend to a large extent on the locations at which juveniles mature. For example, juveniles residing near the Labrador coast almost certainly join the Labrador population. But some juveniles spawned on the Labrador shelf might also drift southwards and join the cod population on the northeastern Newfoundland shelf. This model predicts that genetic variation would be higher in southerly populations because drift only occurs southward. Juveniles would be predisposed to learn migration routes from older fish. Once mature, cod would home to spawn to the same region each year by piloting (the use of remembered features and landmarks; Griffin 1955). This model is fully consistent with the tagging and other historic data and descriptions of the stock distribution on the northeastern Newfoundland and Labrador shelves.

Data required to test these alternative models are not available at present. However, we suggest that a better understanding of the stock structures and migratory life histories of cod in Newfoundland waters, and elsewhere, rests on finding answers to some of these fundamental ecological questions.

Changes in Population Distribution

An examination of the trawl survey data collected during the fall by the Department of Fisheries and Oceans (Baird et al. 1992) suggests that the stock structure evident historically breaks down after 1988 (Fig. 2). Prior to 1988, the proportions of year classes found in 2J, 3K, and 3L remained relatively stable over the life of most of the year classes (Fig. 3). This stability adds further support to the historical interpretation of the stock structure. However, from 1988 to the present, an increasing proportion of the biomass has become concentrated in the southern regions (Fig. 2). Distribution changes are especially evident for older fish. For example, the 1981 year class decreases abruptly in 2J in 1988 and increases unexpectedly in 3K after 1989 (Fig. 3). Similar changes can be observed for the 1980, 1982, 1983, and 1984 year classes (ages 8, 6, 5, and 4,

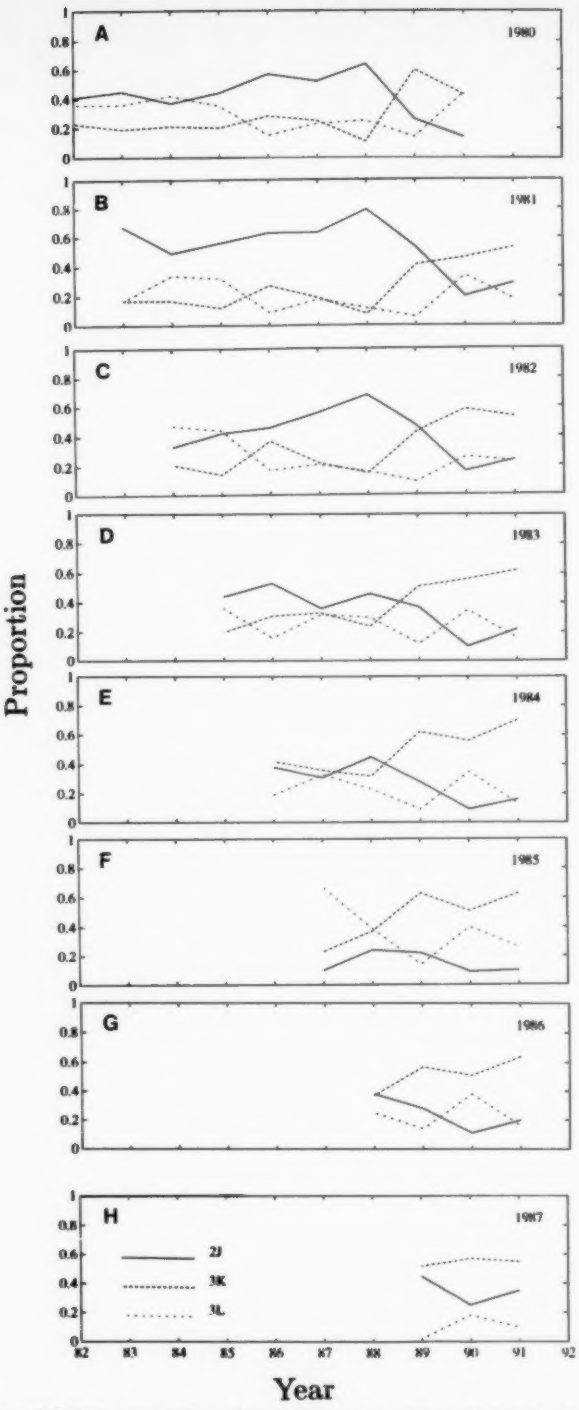

FIG. 3. Relative proportions of year classes spawned from 1980 to 1987 in NAFO divisions 2J, 3K, and 3L, with the year effect removed. Data are from the fall research surveys (Baird et al. 1992).

respectively). There is less evidence for redistributions of the 1985–88 year classes (ages 4 and younger).

A key question is whether the distribution changes were caused by fish movements (migratory model) or were simply a consequence of differential fishing mortality (nonmigratory depletion model). The nonmigratory model indicates that cod populations are spatially fixed and were simply "fished-down" from north to south. We believe that fishing precipitated and continues to contribute to the rapid decline in cod abundance in Newfoundland waters in the past decades. However, the disproportionate decline in abundance in the Labrador region (2J) is unlikely to have been caused solely by fishing. Total losses to fishing were greater in the southern regions than in 2J in every year since 1982 (Baird et al. 1992). Moreover, unregulated foreign catches were concentrated in the most southerly part of the range during the 1980's and early 1990's. Hence, fishing mortality was likely higher in the south than in the north during this period (Fig. 4). It is conceivable that fishing was responsible for high levels of mortality of the redistributed fish in the southern regions from 1989 onwards. It now appears that most of these emigrants have perished.

We attribute southward migrations of cod to declining sea temperature (Rose et al. 1994). However, it should be kept in mind that temperature may be a proxy for other physical parameters of the ocean (e.g., sea-ice extent) and even biological variables, many of which are correlated at long time scales. For example, capelin distributions (*Mallotus villosus*), like those of cod, appear to have shifted southwards since 1989 (J. Carscadden, Department of Fisheries and Oceans, P.O. Box 5667, St. Johns, NF A1C 5X1, Canada, personal communication). Capelin is the chief food of cod in Newfoundland waters (Lilly 1987), and food shifts influence cod distributions (Beverton and Lee 1965; Rose and Leggett 1990). Thus, deteriorating food supplies for cod in the north could also be a key factor in their movements.

We argue that the stock structure of cod on the northeastern Newfoundland and Labrador shelves could not be considered to be stable except at short time scales (much less than a decade). Spatial patterns and richness (Sinclair and Iles 1989) are regulated by the vagaries of environmental conditions (Iles and Sinclair 1982; Sinclair 1988). Historically, cod populations that formed the "stock complex" (Harris 1990) were likely distributed on many banks from northern Labrador to the Grand Banks. Each "population" was sustained as long as conditions permitted. Smaller populations may have been severely reduced by overfishing. Specific adaptations (genetic) and behaviors (learned) developed to maintain stock and migration patterns (at present, we know little of these mechanisms). In the past three decades, environmental conditions have become less hospitable to cod in the northern reaches of their range in Newfoundland waters. Fish removed first by overfishing and then migration have not been replaced by new recruits. Thus, the more northerly systems have not sustained their once abundant cod populations. Spatial variability in cod populations in northern oceans is not considered unique to Newfoundland waters. Major shifts on cod distributions have been well documented from Greenland and Icelandic waters (Horsted 1989; Hovgård and Buch 1990).

It is noteworthy that our interpretation of stock structures in Newfoundland waters follows the approach of McCall (1990) who proposed that fish populations could become geographically isolated because of varying recruitment success. However, McCall (1990) proposed that fish would redistribute naturally as populations increased. Our model differs in that it suggests that density-independent redistribution occurs in response to changing ocean conditions (Sinclair and Iles 1989).

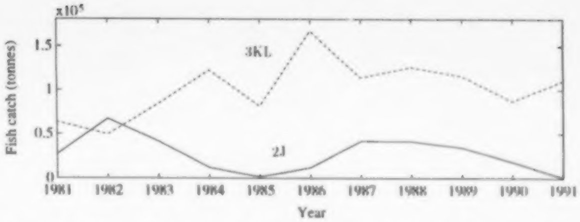

FIG. 4. Offshore fish catch in NAFO divisions 2J (solid line) and 3KL (broken line). Data are from Baird et al. (1992).

The historical fishery in Newfoundland and Labrador was characterized by remarkably stable catches and also by a large degree of spatial variability (Harris 1990). In particular, the Labrador fishery fluctuated at long time scales (Anonymous 1992). We believe that the overall catch stability was a consequence of large cod populations and the low fishing mortalities of that era. Population fluctuations may not have been detected by the fishery. However, spatial variations were noticed then as now. This is consistent with our model of cod stock dynamics in this region. Accordingly, at times when conditions are best in the north, populations levels will be highest and spatial variations in catch lowest. When conditions are poor in the north, population levels will be poorest and spatial variations in catch highest.

Recruitment

The current paradigm is that cod from northern Labrador to the northern Grand Bank recruit as a large-scale unit and that smaller scale spatial processes are not important. Koslow et al. (1987) suggested large-scale synchrony of cod recruitment in the Northwest Atlantic. However, more recently, Cohen et al. (1991) reported that the dominant factors for recruitment of cod from Georges Bank to the Labrador shelf operate on more local scales and that recruitment correlations are strongest for neighboring populations. However these data are analyzed, there is evidence of synchrony at large scales but there is also variability at smaller scales. Myers et al. (1993) calculated recruitment separately in regions 2J, 3K, and 3L of Newfoundland waters and while there is some correlation between them, there are also significant differences. Unfortunately, the analysis of Myers et al. (1993) is based on survey data collected in the fall, when the cod populations overlap in distribution; hence, there is some doubt about the independence of recruitment estimates of these separate stocks based on these data. We argue that recruitment varies at smaller scales in response to physical and biological characteristics of specific regions. Here, we test the relationship between available pooled recruitment estimates for 2J3KL cod and physical environmental factors in an effort to shed light on environmental links to ecosystem and population dynamics.

Exploring ocean parameters for causes of recruitment variations is difficult (Shepherd et al. 1984) and there is need for caution. Lapointe et al. (1992), for example, showed the sensitivity of the recruitment estimates derived from the virtual population model. There is evidence that spurious correlations may occur between recruitment and environmental variables because of the influence of the natural mortality term in the

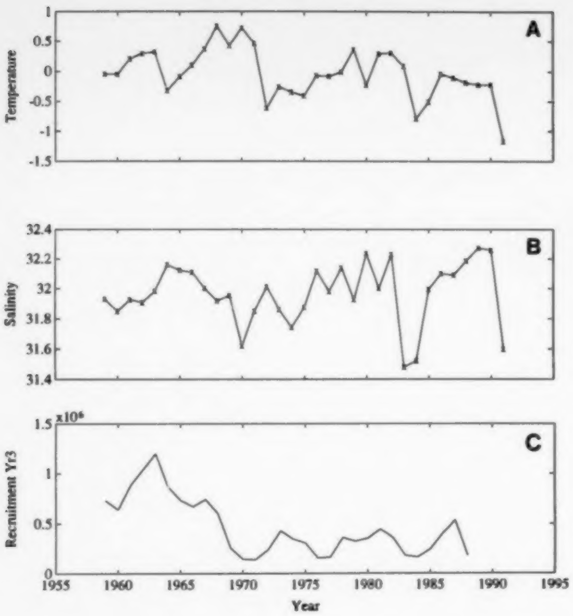

FIG. 5. (A) Temperature and (B) salinity vertically averaged from Station 27 on the inner part of the northeastern Newfoundland shelf (Fig. 1A), with the seasonal signal removed. (C) Recruitment of 3-yr-old cod for the full 2J3KL stock from sequential population analyses (from Baird et al. 1992).

virtual population model (Lapointe and Peterman 1991). In spite of these concerns, we shall explore the best available data, here recruitment estimates from a model analysis of the data.

Temperature

Studies of interannual variability in the Northwest Atlantic are, for the most part, still at the correlative stage. Large interannual variations in several parameters (e.g., air temperature, water temperature, salinity, wind stress, atmospheric pressure, sea ice concentration) have been observed (cf. Petrie et al. 1992) although the nature of their interactions and the processes involved remain obscure. Some specific process models have been developed and applied to this region. For example, Ikeda et al. (1988) used a two-layer model to show that interannual variations in the position of the ice edge on the Labrador shelf could be related directly to meteorological forcing, and Petrie et al. (1992) demonstrated that wind and air temperatures explain about 50% of the variance in the interannual sea temperatures. In spite of these examples, we still lack a model of the relationship between the primary physical variables of interest.

The difficulties of the correlative approach are evident in an examination of the time series of recruitment of 2J3KL cod (Baird et al. 1992) and temperature and salinity at Station 27

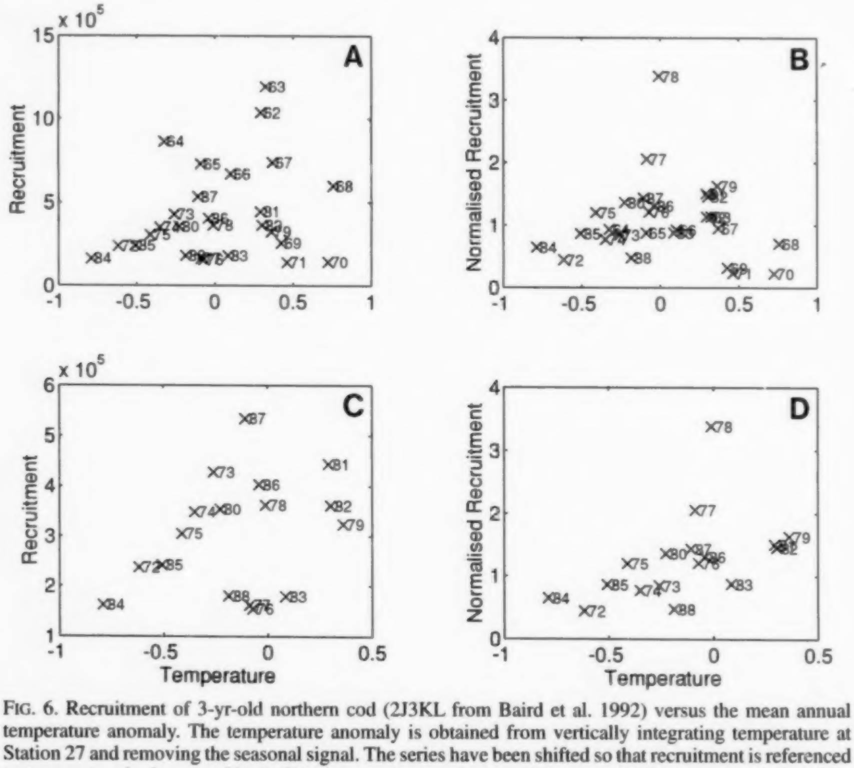

FIG. 6. Recruitment of 3-yr-old northern cod (2J3KL from Baird et al. 1992) versus the mean annual temperature anomaly. The temperature anomaly is obtained from vertically integrating temperature at Station 27 and removing the seasonal signal. The series have been shifted so that recruitment is referenced to temperature in the year of birth. We plot recruitment of year 3 fish as (A and C) numbers and (B and D) numbers normalized by the number of 6+ fish. In Fig. 6C and 6D, we have removed the pre-1972 data.

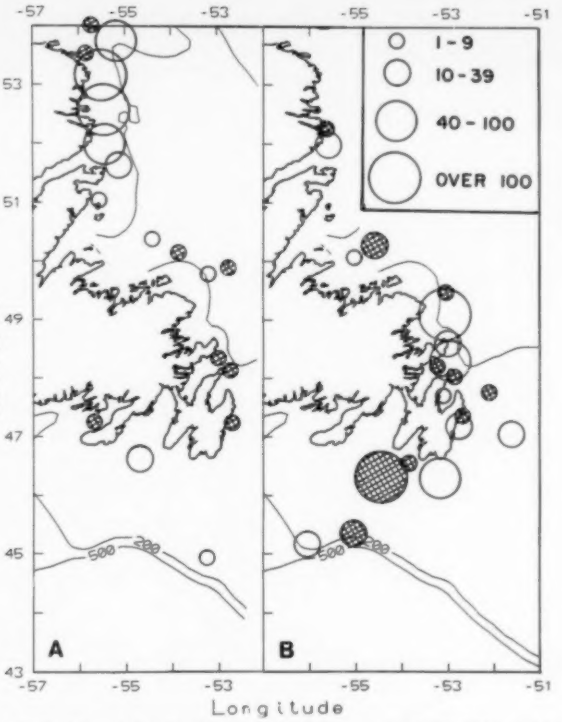

FIG. 7. Cod larval distribution in (A) 1934 (warm) and (B) 1935 (cold) (redrawn from Frost 1938). Open circles are from the spring sampling; cross-hatched circles are from the fall sampling.

(Petrie et al. 1992) on the inner part of the shelf near St. John's (Fig. 5). Correlations may be found between these series (p's < 0.05), but it is difficult to interpret lagged correlations in short time series dominated by low-frequency signals. For example, Petrie et al. (1992) showed that air temperature, water temperature, air pressure, wind stress, and sea-ice extent are all correlated on the Newfoundland shelf. Analyses of the recruitment data reveal significant correlations at different lags (e.g., Sutcliffe et al. 1983). However, there are only a few cycles of each series during the roughly 30-yr record. The probability of spurious correlations is high. The declining trend in water temperature during the past 30 yr evident at Station 27 (Fig. 5A) is related to other large-scale phenomena. Recent analysis of atmospheric and sea-ice data clearly shows a −0.25°C per decade change in Northwest Atlantic temperatures over the last 30 yr (Chapman and Walsh 1993). This decline is a northern anomaly. Temperature is increasing over the North American continent and also over the Northeast Atlantic but appears to be decreasing in waters from Greenland to Newfoundland.

An alternative approach in the analysis of environment and recruitment is to present these data as a scattergram (Ellersten et al. 1989; Loeng 1989). This approach shows that at low temperature, recruitment is always poor (Fig. 6). At high temperatures, no relationship between recruitment and temperature is apparent. If the recruitment data are normalized against stock size, some points change relative position (see Fig. 6B and 6D) but the relationship between cold temperature and poor recruitment remains. We have normalized by spawning numbers (6+ fish) because of an apparent stock–recruitment relationship. Because of incomplete and varied sampling techniques used to collect fish data prior to 1972, there are strong reasons to doubt the quality of these data (Baird and Bishop 1986). We have replotted the recruitment versus temperature anomaly scattergrams without the pre-1972 data to explore the possible influence of these data. Although there are differences between Fig. 6A and 6C and Fig. 6B and 6D, all of the plots show that recruitment is generally low when the temperature anomaly is strongly negative. We do not believe that these results reveal a simple linear relationship between recruitment and temperature, such as might be verified by a regression analysis. Instead, we interpret these scattergrams as showing that temperature acts as a threshold variable for recruitment. Others have carried out regression analysis between recrutment and salinity to show that there may indeed be significant correlations with salinity (Sutcliffe et al. 1983; Myers et al. 1993). Loeng (1989) has shown that Barents Sea cod show similar poor recruitment when their environment is strongly affected by inflows of cold Arctic water (he does not consider a correlation with salinity).

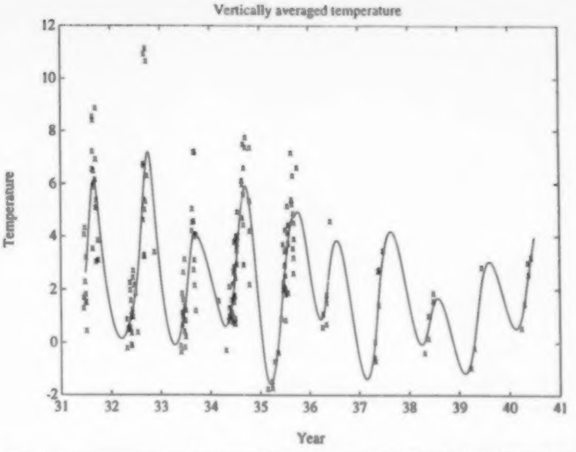

FIG. 8. Temperature on the northeastern Newfoundland shelf. The data shown with crosses have been vertically averaged and are plotted together with a splined smoothing curve.

Although the relationship between cold temperature and poor recruitment is not compelling evidence of a causal connection between these factors, we believe that it points to a link between ecosystem changes and population responses. We believe that cold ocean temperatures influence the spatial patterns of cod movements, spawning locations, and the resultant survival of their progeny. We note that previous workers have attributed the cold water – poor recruitment relationship to temporal matches of spawning with food and ice. For example, the Barents Sea cold water – recruitment relationship was proposed to reflect physiological stress and a poor food supply at low temperatures (Ellersten et al. 1989; Loeng 1989). Myers et al. (1993) explored the idea of food limitation (Sutcliffe et al. 1983), but concluded that the pertinent salinity variations were negatively correlated with phytoplankton abundance. Other temporal links, e.g., salinity and sea-ice formation, which could cause larval freezing, were also discounted by Myers et al. (1993).

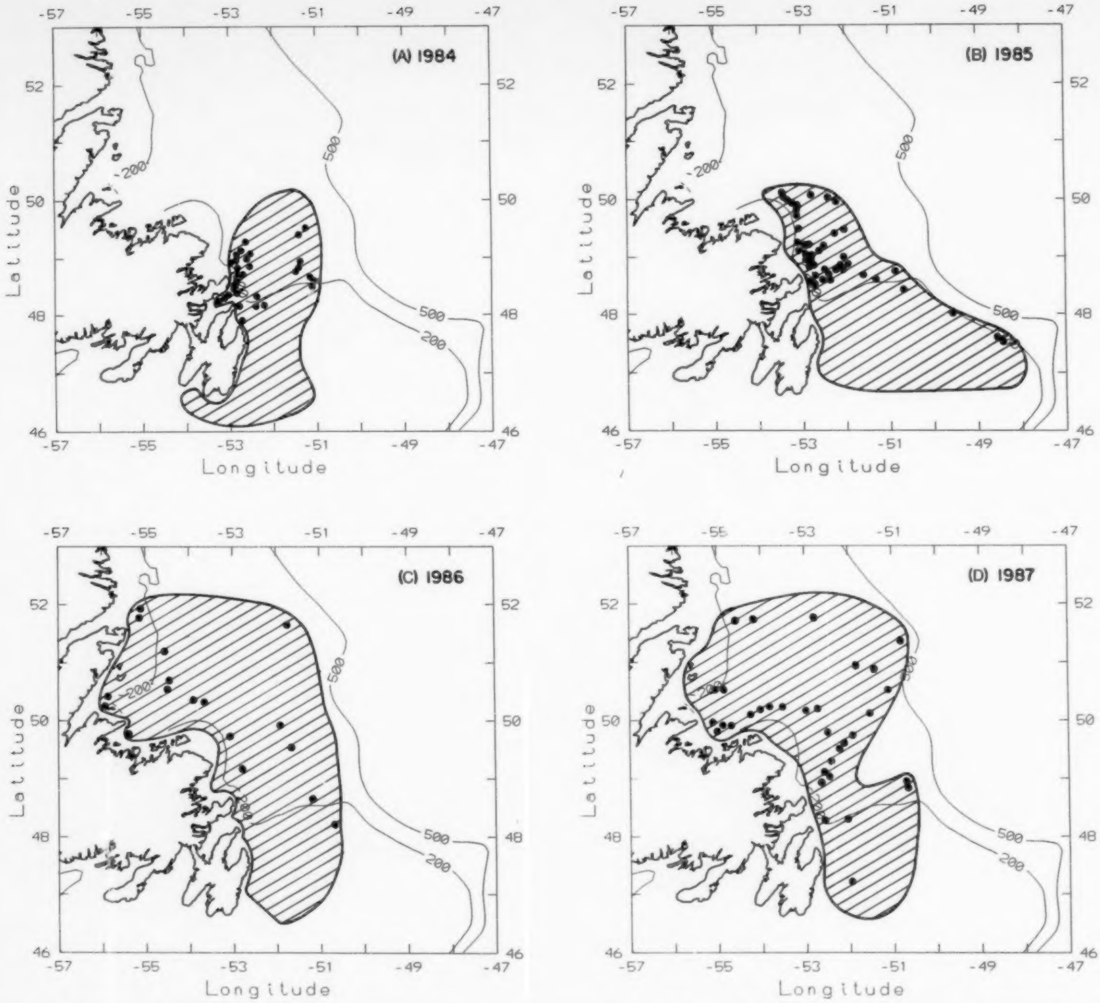

FIG. 9. Spring (early to mid-June) distribution of cod on the northeastern Newfoundland shelf assessed with echo sounders. 1984 and 1985 were cold years (see Fig. 5) with poor recruitment and 1986 and 1987 were warmer years with stronger recruitment (see Fig. 6). The hatched areas are the surveyed areas; each circle represents the occurrence of a cod aggregation with dimensions >1 nautical mile.

Stock Spatial Dynamics

We propose that the link between thermal conditions and recruitment success in the northern cod is less direct than proposed by Loeng (1989) for the Barents Sea and lies in the spatial dynamics of spawning relative to the stock range and the environment. Recruitment may be determined as much by the relative distribution of the fish as by their absolute abundance. For example, in the dynamic Labrador and northeastern Newfoundland shelf current system, egg and larval drift and probable settling sites will not be independent of the location of spawning. Northern and shoreward spawning would be expected to be more successful than seaward and southward spawning because of the drift patterns for eggs and larvae on the shelf (Helbig et al. 1992). Could a link between cold temperature and poor recruitment be found in the distributions of spawning adults? We examined several data sources to explore this hypothesis. The first was cod egg and larval distribution data (Fig. 7) reported for the early 1930's (Frost 1983) combined with oceanographic and fishery data reported by Thompson (1943). These data suggested that interannual variations in the egg and larval distribution patterns along the Labrador and Newfoundland coast were associated with variable thermal conditions on the northeastern Newfound shelf. In 1935, water temperatures were much colder (<0°C) than in 1934 on the Newfoundland shelf (Fig. 8). At the time, thermal conditions were believed to be associated with the relative strength of the cold-water Labrador current (Thompson 1943). In 1934, a relatively warm year, cod larvae were concentrated in the northern portion of the range off Labrador. Although no comprehensive data on adult cod distribution are available for this era, trap fishery catches, which reflect overall available biomass (Rose and Leggett 1988, 1989; Rose 1992), were recorded in detail. In 1934, the spring trap fishery was strong in northern Labrador and weak in southern Labrador. Similarly in 3K, the trap fishery was strongest in the north and failed entirely south of Cape Bonavista. This pattern in the trap fishery suggests that adult cod were distributed in the postspawning period in the northern parts of their ranges in the warm year 1934. This pattern changes abruptly in 1935, a cold year in which larvae were concentrated in the southern ranges off eastern Newfoundland. Thompson (1943) interpreted these larval distribution data as reflecting the stronger flows of the Labrador current in 1935 which could have carried eggs and larval further southward, but there is no evidence that cold years and strong flows are correlated. We suggest that differing spawning distributions may have played a large role in producing these very different larval distribution patterns. As evidence of a more southerly spawning distribution in 1935, the best fishing was found "well to the south — e.g. at Bay Bulls (on the east coast of the Avalon Peninsula), the water temperature being too low further north in this cold year" (Thompson 1943).

We also examined fisheries acoustic data collected by the Department of Fisheries and Oceans describing spring (post-spawning) cod distributions on the northeastern Newfoundland shelf for 1984–87 (Fig. 9). These years included two very cold years, 1984 and 1985, with poor recruitment and two warmer years with strong recruitment (at least in 3K). We found that in the colder years (1984 and 1985), cod were relatively abundant in the southern reaches of their range in early June (the northern reaches could not be surveyed because of ice conditions). During the warmer years (1986 and 1987), cod were distributed widely in the northern portions of their range at the same time of year and were less frequently observed and less widely distributed in the southern ranges than in 1984 and 1985. Differences in overall abundance could not account for the observed pattern differences because total biomass (>>3 yr) differed by only 20% and the biomass of older fish (>>7 yr) did not differ between these years (Baird et al. 1992). Note that sampling was not equivalent in all years, and we do not know where spawning occurred. Still, these data add support to the hypothesis that in cold years, cod will be found in more southerly locations.

Examination of offshore fishing success from 1981 to 1986 when fishing distributions were uncontrolled indicates that catches peaked in 2J in 1982 (Fig. 4) when ocean conditions were warm and strong recruitment prevailed (Fig. 5) and then declined during 1984–85 (cold water and poor recruitment), rebounding in 1986. The opposite pattern is evident in catches from 3K. Although these patterns may be partially driven by fleet dynamics and ice conditions, it seems unlikely that fishermen would bypass large concentrations of cod in 3K to fish in the more distant 2J zone. An alternative interpretation is that the major cod concentrations were not present in 3K during the winter months until 1984. We note that interpretation of the catch data (Fig. 5) since 1987 is confounded by the imposition of controls on fishing distribution beginning that year.

To further investigate a possible spatial basis for the recruitment–temperature link, we examined distributions during the 1960's when recruitment was on average double the levels observed during the 1980's. It was obvious that the higher levels of recruitment during the 1960's were associated not only with more abundant stocks but more northward distributions of cod (Harris 1990) which in turn coincided with warm ocean conditions (Fig. 5). Were the high levels of recruitment during this era solely the result of the high abundance levels? Or did the northward distribution and spawning of the stocks during the 1960's (Postalakii 1972) enhance recruitment success across the full range of the stock and hence help sustain high levels of abundance, at least for a time, even in the face of heavy fishing mortality imposed by European-based trawlers?

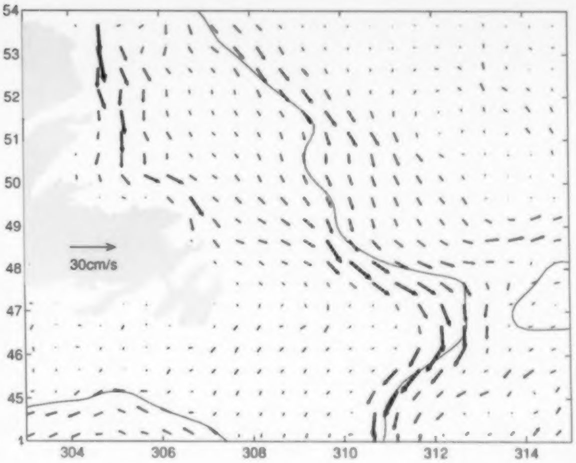

FIG. 10. Spring surface circulation for the northeastern Newfoundland shelf derived from a diagnostic model applied to the northeastern Newfoundland shelf (deYoung et al. 1992). This plot shows the inshore branch of the Labrador current, on the inner part of the shelf, and the outer branch, located at the shelf break (see Fig. 1B). The 500-m isobath is indicated by the solid line.

The weight of the evidence suggested the hypothesis that cold water temperatures might influence cod spawning locations. If this is true, then it is likely that the variations in distribution result from cumulative exposure to thermal variations over the previous spring and fall. At these times, cod undertake cross-shelf migrations to feed (spring) and to return to the offshore wintering grounds (fall) (Templeman 1966; Rose et al. 1991). Hence, during these travels, fish are directly exposed to any interannual variations in seasonal temperature signals. Our view differs substantially from the cold-water barrier hypothesis (Templeman 1966; Lear et al. 1986) that attempts to explain variations in the migration patterns and inshore catches of cod. We propose that at the large-scale, cod respond to the cold water after integrating over a migration cycle. Thus the fish migrate through the cold intermediate layer, but redistribute southwards before the next spawning season in response to this cold water and in search of warmer conditions in which to mature. Thus, they move south because there is warmer water across the shelf to the south.

Cod are highly sensitive to small temperature changes (Harden-Jones and Scholes 1974), although they are able to produce an antifreeze protein that enables them to adapt to cold water (Goddard et al. 1992). The spatial response would be to interannual rather than immediate conditions and could be mediated by growth and maturation cycles. Thus, variability in thermal conditions on the shelf could be reflected in spawning locations even though waters having temperatures for spawning (3–5°C) occur all along the shelf edge at depths greater than 300–400 m. Dadswell et al. (1987) postulated a similar thermal mechanism for migrating American shad (*Alosa sapidissima*). However, if northerly spawning is critical for good recruitment in northern cod and spawning occurs along the shelf edge, where water depths are 300–500 m (Fig. 1B) and temperatures are ≥3°C even in cold years, then why do cod not just move north to spawn along the shelf edge even in cold years? The answer may

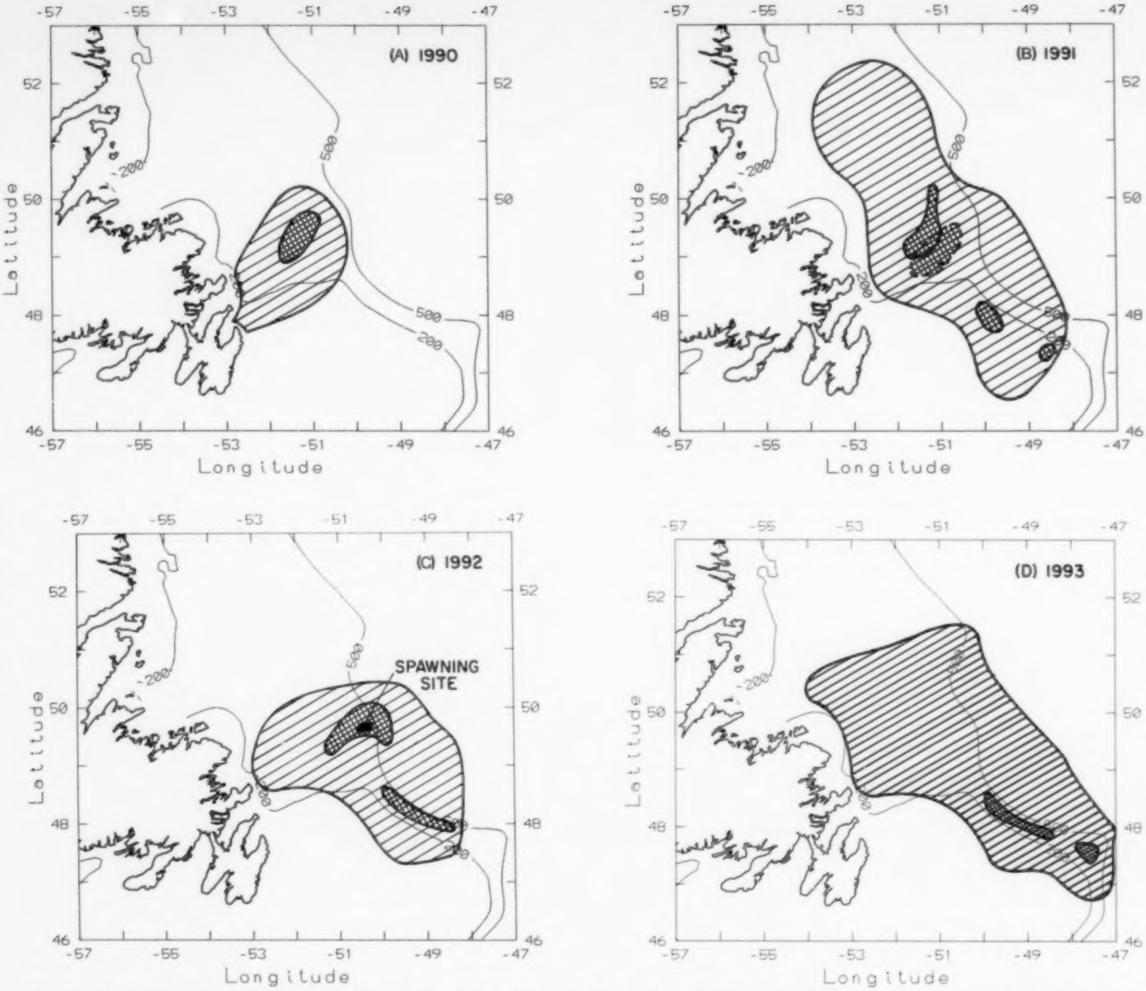

FIG. 11. Maps of the (A) 1990, (B) 1991, (C) 1992, and (D) 1993 generalized spring distributions of adult cod on northeastern Newfoundland shelf. Cross-hatching indicates where fish were acoustically detected and biologically sampled within the overall survey area (hatched). The site where spawning was observed in 1992 is marked.

be that a major portion of cod spawning occurs shoreward of the shelf break where waters are more variable relative to the interannual signal than at the shelf break itself. Shoreward temperatures may at times be much cooler than cod prefer in the prespawning and spawning period. It is noteworthy that recent observations have shown that cod do spawn well shoreward of the shelf break in waters with temperatures of 2.5°C (Rose 1993). Analyses of historical distribution patterns also indicate that cod may spawn at locations well shoreward of the shelf break (Hutchings et al. 1993).

Right Site Hypothesis

We propose that the distribution of spawning cod on the northeastern shelves plays an important role in determining recruitment; we call this dependence the "right site" hypothesis. This is a variant of the spatially based recruitment model advanced by Iles and Sinclair (1982). We suggest that in cold years, cod on the northeastern Newfoundland and Labrador shelves are likely to be distributed in the southerly portions of their ranges. A southerly distribution pattern and spawning would result in short residence times for eggs and larvae and a decreased probability of larval settling in a favorable area on the shelf (Helbig et al. 1992). Hence, cold years would be unlikely to yield high levels of recruitment. In warm years, spawning would take place in the more northerly reaches of the range. Hence, warm years would result in longer egg and larval residence times on the northeastern Newfoundland shelf and higher probabilities of larvae being advected shoreward to a favorable settling ground. On the northern Grand Banks, spawning sites in cooler years would likely be distributed eastward to deeper warmer waters at the eastern edge of the Grand Banks. In warm years, these fish would spawn on the northwest edges of the Bank in shallower waters. It follows that during warm years, there is the potential for much higher recruitment at the egg and larval stage than in cold years for all populations of Newfoundland cod.

Gadoid stocks have been shown to occupy an area in rough proportion to their abundance (Crecco and Overholtz 1990; Gordoa and Duarte 1991; Rose and Leggett 1991). The densities of spawning aggregations have been observed to be relatively constant on the northeastern shelf (G. Rose, unpublished data). Hence, northward distributions and spawning are likely to be facilitated not only by warmer ocean conditions but by high levels of abundance which cause cod to be distributed over a wider area. By chance alone, a more abundant stock is more likely to spawn at the right site. In contrast, a smaller stock is more likely to have to put all its eggs in one basket by spawning in a contracted spawning area. This effect would compound the influence of stock size on recruitment.

Egg Dispersion

Cod eggs are released at the bottom, rise to the surface, and mature in about 30–40 d at temperatures typical for their historical spawning areas, from the north end of the Grand Banks to the north end of the Labrador shelf (Fig. 1B). The exact time at which the larvae settle to the bottom is not known but larvae are in the surface waters (0–50 m) for at least several weeks after hatching. Helbig et al. (1992) developed a model of cod egg dispersion on the Newfoundland shelf driven by a simplified circulation field and meteorological forcing. The primary circulation field in this region consists of mean southward flow made up of the inner and outer branches of the Labrador current (Fig. 10). Eggs released anywhere on the shelf are likely to be carried southward (Greenberg and Petrie 1988). This conclusion can be drawn from mean current data and is supported by drifter studies on the northeastern Newfoundland Shelf (Venkatesh et al. 1990; Helbig et al. 1992).

The right site hypothesis states that the location of spawning is important to recruitment success. We assume that the longer the eggs can stay on the shelf the greater the probability that they will survive to the larval stage and beyond (deYoung and Davidson 1994). If eggs are carried off into the North Atlantic, then they are lost to the stock (Sinclair and Iles 1989). To test these ideas, we have run advection-diffusion models using the circulation diagram shown in Fig. 10. These studies have shown that residence times are lowest for eggs released at the southern part of the northeastern Newfoundland shelf and near the shelf break. Residence time is highest for eggs released in the northern part of the range and on the inner part of the shelf. Between 48 and 49°N, near the shelf break, there is a southeasterly transport that carries the positively buoyant eggs off the shelf.

There was an intensive effort in 1991 and 1992 to locate cod eggs on the northeastern Newfoundland shelf. The average concentrations during June 1991 over the spawning sites in the northeastern Newfoundland shelf were approximately 1 egg/m^2 (J. Anderson, Department of Fisheries and Oceans, St. John's Nfld., personal communication). On the Scotian shelf, egg densities are roughly 50 egg/m^2 (Hurley and Campana 1989; Brander and Hurley 1992). In Norway, where eggs are concentrated in a coastal region, densities greater than 100 egg/m^2 have been observed (Ellertsen et al. 1981; Sundby 1983). How many eggs do we expect to find on the Newfoundland shelf? If there were 10^8 fish spawning (a minimum number according to the SPA and consistent with a detailed acoustic mapping of the spawning school in June 1992 (Rose 1993)) and each fish lays 10^6 eggs, then over this spawning period there should be roughly 10^{14} eggs released. The surface layer is roughly 50 m deep, the width of the shelf is 200 km, and the length of the sea area potentially holding eggs is given by multiplying the mean current speed of 0.2m/s by the time of spawning, 60 d, to give approximately 1000 km; hence a total area of 2×10^{11} m^2. Dividing the total egg numbers by the volume indicates a concentration of 500 eggs/m^2, much larger than observed. There are, however, many factors that could influence the observed concentration, in particular egg mortality. Nonetheless, these data are consistent with the hypothesis that eggs in the southern region have short residence times on the shelf and are advected off the shelf; hence the low observed concentrations in 1991 and 1992.

There is an indication from these data that egg concentrations are lower than expected on the Newfoundland shelf. Although egg production may be stable over a wide range of spawning-stock biomass, as suggested by Rothschild and Fogarty (1989), egg success can still be an important factor in recruitment. Furthermore, a breakdown in the egg production density-dependent stability may provide a mechanism for a contribution to population collapse (Rothschild 1986). The relationship between egg production and stock biomass for cod is unknown.

Postspawning Distributions, 1990–93

Detailed studies were conducted on spawning and postspawning distributions of northeastern Newfoundland cod in the springs of 1990, 1991, and 1992. Cold conditions prevailed throughout this period, culminating in 1991 when cold waters blanketed much of the shelf. Coincident with these low temperatures, cod were observed to be highly aggregated in the most southerly reaches of their range where warm waters persist (Fig. 11). Cod in spawing condition were located in 1991, 1992, and 1993 in this southern range; a major spawning event was observed in 1992 (Fig. 11C). In 1990 the southerly moving cod had spawned at an unknown but more northerly location. Cod of the Labrador stock declined dramatically during this period. If we are correct in believing that southerly spawning yields poor recruitment, the observed southerly distributions of the stocks in 1991, 1992, and 1993 predict poor recruitment. Thus, based on our model, we suggest that the prognosis for both the Labrador and northeastern Newfoundland cod stocks is poor for at least the next decade.

The Future

We conclude that the current paradigm of the natural rebuilding and redistribution of the "northern" cod is inappropriate. These cod were fished to low levels by the mid-1970's and did rebuild rapidly until the mid-1980's (Harris 1990). What is different now? Although the biomass levels at the start of the moratorium on fishing in the spring of 1992 did not differ greatly from those observed in that earlier era, distribution patterns differed greatly. Through the late 1970's and early 1980's, mature fish were present in relatively large numbers to the north in the Labrador population (2J) (Fig. 2). In contrast, mature fish have been virtually absent there since 1990. Moreover, cod on the northeastern Newfoundland shelf (3K) have become concentrated in the most southerly part of their range since 1989. Hence, we believe that the past will not predict the future because conditions have changed from any previously observed.

A key question for rebuilding the cod stock on the Newfoundland and Labrador shelves concerns the fate of the northern (2J) fish that migrated southward between 1989 and 1991. On the one hand, if migratory behavior and spawning region are genetically determined and the northern fish differ from the more southerly populations on the northeastern Newfoundland shelf (3KL), then perhaps when environmental conditions improve the remnant of the Labrador population will reestablish historic population and migration patterns (assuming sufficient genetic stock has survived). On the other hand, if historic population and migration patterns are geographically and behaviorally based, then it may take several generations and several decades to reestablish migration patterns in the northern regions by recolonization.

In conclusion, predictions of future performance of the northern cod stock based on our model suggest a longer rebuilding time as compared with those of existing models. We offer several specific predictions:

(1) Biomass levels will continue to decline, until a northward shift in distribution occurs, as a consequence of a lack of recruits from the north and continued fishing mortality from foreign fleets.

(2) Regeneration of the northern populations that historically inhabited the Labrador shelf and banks will occur slowly, at scales of decades. Their current numbers represent remnant populations which will take generations and warming ocean conditions to rebuild. There may have been an irreversible loss of genetic material from these populations.

(3) Regeneration of the present southern populations will occur much more quickly given warming ocean conditions which will enable these fish to spawn to the north (as in the 1960's) and at more shoreward locations.

(4) The 1991, 1992, and 1993 year classes will likely be poor in abundance because of cold temperatures and the southerly distributions of cod. We believe that the year classes will be even smaller than would be predicted from the stock–recruitment relationship and the low stock numbers.

(5) Although oceanographic predictions are somewhat unreliable, present indications are that we are enduring a period of local cooling in the North Atlantic (Chapman and Walsh 1993); hence, we can expect continued poor recruitment until stock range and biomass expand to the northerly areas. Improvement in this prognosis will be heralded by a northward and shoreward shift in distributions from the present southerly and seaward pattern.

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REVIEWS/SYNTHÈSES

Management of the 4WX Atlantic Herring (*Clupea harengus*) Fishery: An Evaluation of Recent Events

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The Atlantic herring (*Clupea harengus*) fishery of the Scotian Shelf and Bay of Fundy regions of Canada (NAFO Divisions 4W and 4X) is pursued with multiple gear types including traps, gillnets and purse seines, which have evolved in importance over time with changing market emphasis. It was one of the first commercial fisheries to be regulated by limited entry (since 1970), and in 1972 it was the first to come under nationally allocated annual total allowable catch (TAC) limits. In 1976 an individual vessel quota scheme for purse seiners was established and operated jointly by the regulator and the harvesting sector. That initiative formed the basis of the 1983 10-Year Management Plan for 4WX herring that is reviewed and evaluated in this paper. The Plan, while not expressly achieving its original goals, and not fully realizing the implicit benefits of an individual transferable quota (ITQ) system, has contributed to the continuation of consensus management of this, the only large Atlantic herring fishery which has not suffered a major collapse. The development of successful management plans in the future must deal explicitly with ongoing problems of unstable markets for herring products and under-reporting of catches.

Au Canada, la pêche au hareng atlantique (*Clupea harengus*) dans les régions du plateau Scotian et de la baie de Fundy (divisions 4W et 4X de l'OPANO) se fait avec de nombreux types d'engins, notamment les casiers, les filets maillants et les sennes, dont l'importance a varié avec le temps, selon les conditions changeantes du marché. Il s'agit de l'une des premières pêches commerciales à être réglementée par un nombre limité d'inscriptions (depuis 1970); en 1972, c'était la première à être soumise à un régime national déterminant un total annuel des prises admissibles. En 1976, un régime de contingents individuels par bateau, applicables aux senneurs, a été créé et géré conjointement par le législateur et les pêcheurs. Cette initiative constitue le fondement du plan de gestion décennal de 1983 pour le hareng 4WX, qui est examiné et évalué dans cet article. Même s'il n'atteint pas expressément ses objectifs initiaux et qu'il ne livre pas tous les avantages implicites d'un régime de contingents individuels transférables, le Plan a favorisé la continuation de la gestion consensuelle de la seule grande pêche au hareng atlantique qui n'a pas subi un effondrement. L'élaboration future d'un plan de gestion efficace doit traiter explicitement des problèmes actuels qui sont liés à l'instabilité des marchés pour les produits du hareng, ainsi qu'à la sous-déclaration des prises.

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The 4WX (Scotian Shelf, Bay of Fundy) Atlantic herring (*Clupea harengus*) fishery is an interesting and important case study in fisheries management. It is the largest herring fishery in the western Atlantic, with recent landings in excess of 100 000 t annually. The commercial fishery involves a variety of gear types including weirs (one of North America's oldest trap fisheries), shutoffs (use of seines to trap fish in small coves), and gillnets, and is dominated by a purse seine fleet of 40 vessels. The fishery has survived major changes in market emphasis and demand; and has been dominated at different times by sardine (juvenile herring), meal, fillet and roe markets. This herring fishery has been a model in the development of innovative fisheries management: it was one of the first fisheries managed under limited entry (since 1970), and it was the first to come under nationally allocated annual exploitation limits or "TACs" in 1972. There has been considerable involvement of the fishing industry in the management process through bodies such as the Atlantic Herring Management Committee established in 1972, and its successor since 1981, the Scotia–Fundy Herring Advisory Committee. Most significantly, in 1976 the herring purse seine fishermen agreed to an individual vessel quota scheme. The quota program was managed jointly by the Department of Fisheries of the Canadian government and the herring harvesters themselves. It is the first known application of individual vessel quotas in a commercial marine fishery. An individual quota system later formed the foundation of the 10-Year 4WX Herring Management Plan of 1983, a major attempt to restructure the fishery that established guidelines for suballocation of TAC among gear sectors and transfers of individual quotas among purse seiners. Indeed, this fishery is often cited as a case study in the application of individual transferable quotas (ITQs) and property rights in fisheries (see, for example, Campbell 1981; Doucet 1981; Mollet 1986; Geen and Nayar 1989; Muse and Schelle 1989; Pearse 1991).

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Although the events leading up to, and circumstances surrounding, the development of management measures in this fishery have been recorded in a variety of reports (Peacock and MacFarlane 1986; MacFarlane 1989; Iles 1993), there has been no detailed account of recent events and little evaluation of the results of management initiatives, particularly those of the 1983 10-Year Plan. The purpose of this paper is to fill this gap and thereby to provide a basis for evaluation of the 10-Year Management Plan and discussion of future management. Implicit in the 10-yr strategy were the perceived benefits to have been derived from quasi-property rights (Neher et al. 1989) underlying the allocation of individual transferable quotas. The experience of the management of the 4WX herring fishery provides the earliest experiment in ITQ management in a commercial marine fishery. This paper presents an evaluation of this strategy.

The 4WX Herring Stocks

Management of 4WX herring is based on the biological stock, and requires definition of spatial boundaries and quantification of abundance. The following section provides a brief introduction to the structure of the herring stocks in this area and the established procedures for assessing the size of this resource.

Stock Structure and Management Unit Definition

Atlantic herring spawn at discrete locations and at predictable times. The attributes of the recurring spawning groups are such that the species is commonly believed to return to sites of previous spawning — and likely to "home" to the spawning area of origin. As such, spawning groups of herring are considered to be distinct populations, and although the scale and degree of discreteness can be debated (Stephenson 1991a), this model forms the basis for stock assessment and management.

The Bay of Fundy/Gulf of Maine and Scotian Shelf area contains several major herring spawning sites (Fig. 1), plus a number of smaller (mostly coastal) sites. These spawning groups exhibit a range in spawning time (May–November), population size (including the largest in the northwest Atlantic) and physical conditions of the spawning area (depth, substrate, etc.) generally typical of Atlantic herring.

Although herring migrate extensively and mix as juveniles and adults, major patterns have been elucidated from extensive tagging studies of the 1970's (NAFO 1983), a variety of stock identification studies (Sindermann 1979) and biological information from catch data in the fishery. As a result, herring spawning groups of the Scotian Shelf, Bay of Fundy and Gulf of Maine have been aggregated into three geographical units, namely NAFO Divisions 5Y, 5Z, and 4WX, for assessment and management (Fig. 1). These recognize, as far as is practical, the assumption of biological discreteness (particularly for major spawning groups) – but also reflect political boundaries (Canada/USA and 200 mile jurisdiction) and historical management unit definitions.

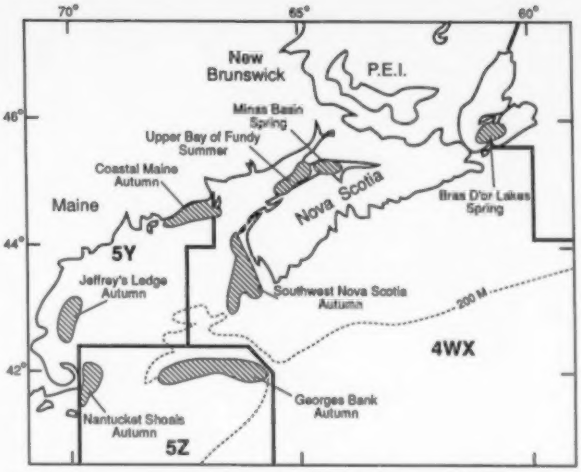

FIG. 1. Management units and major spawning areas of Atlantic herring in the Bay of Fundy, Gulf of Maine, and Scotian Shelf.

The current 4WX management unit remains largely as it was established by the International Commission for the Northwest Atlantic Fisheries (ICNAF) during the early 1970's (ICNAF 1976). It is dominated by fish that spawn off southwest Nova Scotia (late summer and autumn spawners) and are believed, from tagging results, to overwinter off eastern Nova Scotia (Cape Breton); but also includes a number of other spawning areas, including upper Bay of Fundy spring and summer spawners (Fig. 1). Traditionally, the scientific assessment has considered the stock origin of fish taken in each fishery within the management unit and has made corrections for obvious migration between management units, the most significant of these being the exclusion of juvenile herring taken in New Brunswick weir and shutoff fisheries which are considered to be migrants from the Division 5 management units (Stephenson et al. 1991).

The distribution of commercial fishery effort broadly reflects the seasonal movements and distribution of fish, but is modified substantially by market demand, weather, regulation, and gear limitation. Adult herring are taken by purse seine in prespawning aggregations, primarily off Southwest Nova Scotia and in the Bay of Fundy in early summer, then by purse seine and gillnet on more specific spawning grounds in late summer/autumn (Fig. 2). A winter purse seine fishery takes place on what is considered to be the major aggregation of these fish after migration to eastern Nova Scotia. Juvenile herring have traditionally been taken by weirs at the mouth of the Bay of Fundy (especially on the New Brunswick shore) from spring through autumn, and to some degree by purse seine and midwater trawl in the same area in winter.

Stock Assessment

4WX herring has been assessed annually in recent years by the Canadian Atlantic Fisheries Scientific Advisory Committee (CAFSAC). The framework for the assessment has been an age

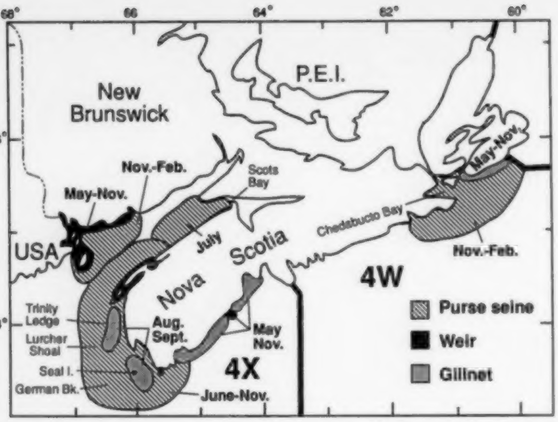
68°
66
64°
62°
60°
46°
44°
New Brunswick
P.E.I.
Nov.-Feb.
Scots Bay
May-Nov.
May-Nov.
Chedabucto Bay
USA
July
Nova Scotia
Nov.-Feb.
4W
Trinity Ledge
Lurcher Shoal
Seal I.
German Bk.
Aug. Sept.
May Nov.
4X
June-Nov.
Purse seine
Weir
Gillnet

FIG. 2. Spatial and temporal distribution of major gear types in the 4WX herring fishery.

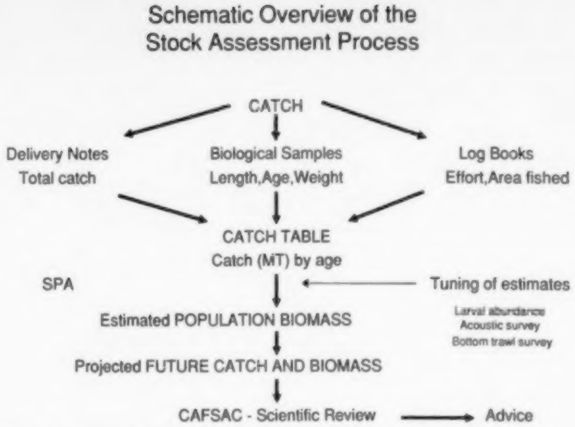
Schematic Overview of the Stock Assessment Process
CATCH
Delivery Notes
Total catch
Biological Samples
Length,Age,Weight
Log Books
Effort,Area fished
CATCH TABLE
Catch (MT) by age
SPA
Tuning of estimates
Larval abundance
Acoustic survey
Bottom trawl survey
Estimated POPULATION BIOMASS
Projected FUTURE CATCH AND BIOMASS
CAFSAC - Scientific Review
Advice

FIG. 3. Schematic overview of the stock assessment process used for 4WX herring.

structured population analysis (Fig. 3) similar to that used for most other Atlantic herring populations (Stephenson 1991b, 1992). Information from the commercial fishery (delivery notes or purchase slips, biological samples and purse seine logbooks) has been used as the basis for an age structured population analysis, calibrated by fishery-independent surveys (Stephenson et al. 1990a, b). Recent analytical assessments have been compromised by misreporting of the commercial catch and by a lack of precision in abundance indices (Stephenson 1992; Stephenson et al. 1992). The traditional calibration for this assessment has been based on an autumn larval herring survey as an indicator of spawning stock biomass, but recent assessments have also used the biomass of overwintering herring from acoustic survey and the occurrence of herring in research bottom trawl surveys (Stephenson et al. 1990a; Buerkle 1991).

Analytical stock assessments indicate a peak in abundance (age 2+) above 600 000 t in the late 1960's, a decrease by approximately half during the 1970's, and an increase during the late 1980's to, or perhaps beyond the 1967 level (CAFSAC 1987, 1988). Projections for this fishery during the early 1980's, under the $F_{0.1}$ target strategy adopted by the Canadian government, indicated that a sustained yield in excess of 100 000 t was possible (Canada 1985), and with the improved stock status during the 1980's the stock was considered to have reached its long-term yield of 150 000 t (Rivard et al. 1988).

Landings from 4WX increased rapidly during the 1960's, with increased fishing power and expanding markets, to peak at almost 200 000 t in 1968, then decreased with management restriction and decreasing stock size to below 100 000 t in 1979. During the 1980's, landings fluctuated between 93 000 and 160 000 t (Stephenson et al. 1991) due to changes in market and availability of fish (relative to market demand for size and condition); however statistics for some of these years were affected by under-reporting (Stephenson et al. 1992).

The Commercial Fishery

Herring Product Evolution

The fate of the commercial herring fishery in Atlantic Canada has been dictated by changing markets for herring products. Herring has always featured in subsistence fishing along Canada's Atlantic coast (Perley 1852), and brush weirs were probably used prior to European colonization (Moore 1898). A commercial herring fishery involving weirs and gillnets began at the turn of the 19th century and supplied fresh fish as well as salted, pickled and smoked herring. Herring was also used locally for bait in other developing fisheries during this time (Perley 1852; Moore 1898). Annual landings for 1850–1900 were in the order of 10 000 t (Kearney 1983).

In 1879 an American consortium perfected a technique for canning juvenile herring as "sardines." The first sardine cannery was established in the Passamaquoddy Bay region, southwestern New Brunswick (Earll 1887) and by 1900, 41 sardine plants were dotted along the coast of New Brunswick west of Saint John, and along the adjacent coast of Maine (Kearney 1983). These utilized predominantly juvenile herring from the weir fishery. Canned sardines formed the backbone of the herring export market for nearly a century. Markets for pickled or "marinated herring" (including rollmops and "Solomon Gundy") and small "bloaters" also emerged during the latter part of the 19th and early 20th centuries. In addition, there was a small traditional local market for cured, smoked herring and for bait.

Between 1900 and 1960 annual herring catches averaged about 25 000 t (Kearney 1983). Weirs accounted for almost all this catch except during World War II when disruption of European fisheries caused a temporary increase in demand for Canadian herring (accommodated largely by the purse seine winter fishery). Seiners were prevented from summer fishing activities through successful government lobbying by weirmen (Doucet 1960).

In the 1960's the use of whole fish for meal and oil emerged amidst an expanding world economy and enhanced technologies for harvesting large quantities of schooling herring using purse seines. Decreases in yield from European herring stocks after 1964–65 (White and Mace 1988), high world fish meal prices and federal government subsidies for the fish reduction industry in Atlantic Canada (Kearney 1983) were additional factors allowing purse seines, the only fishing technique capable of supplying large volumes of fish for reduction purposes, to become the dominant gear.

At the peak of fish meal expansion in 1968, as much as 225 000 t of herring (Kearney 1983) was landed (from 4WX and other areas) to four Nova Scotia and two New Brunswick herring meal plants (Mitchell and McEachern 1970), but purse seine landings to the meal market quickly decreased as a result of low fish meal prices and the imposition of catch limits, resulting in low incomes for the purse seine fishermen.

The difficulties of Atlantic Canada herring fishermen were temporarily solved by poor herring catches in Europe. The collapse of the world's largest stocks of herring in the North Sea in the mid-1970's created considerable demand for Canadian adult herring for food (frozen fillets and blocks), in what had previously been only minor markets. Subsequently, in 1976, the use of whole fish for meal was banned in order to preserve increasingly scarce herring and to persuade fishermen and processors to take advantage of a higher priced herring "food" market in Europe. Financial assistance was provided to herring processors for necessary plant conversions (Newton 1980).

The ban of the directed fishmeal fishery created an oversupply in the market for higher priced food products and created some difficulties as both fishermen and processors adjusted to a new set of market requirements. To ease the transition, an "over-the-side sales" program (OSS) of sale of herring captured by domestic vessel to foreign, in particular East European, processing vessels, was first introduced in 1976 (Kearney 1983).

In spite of claims that OSS could not be used to exert price leverage in domestic, shore-based markets (Kirby 1982), they were introduced to encourage higher prices for herring landed ashore. OSS programs were meant to be an interim strategy to increase fishermen's revenues while the processing sector changed over to the production of food products with assistance of federal government grants and loans (Kearney 1983), but have continued to provide herring fishermen an alternative market for their catches and supplement their incomes to this day.

In the early 1980's, as the North Sea stocks of herring began to recover, Canadian herring exports were gradually displaced but, in 1984, a new market emerged in Japan for herring roe. As part of a general move toward easy to prepare, packaged products, herring roe (kazunoko) began being marketed as a convenience, everyday food known as "ajitsuke kazunoko" or flavored herring roe. This product was developed as a means of utilizing lower quality roe unsuitable for salting.

The emergence of a market for flavored herring roe has been rapid and the single most important factor in the Atlantic herring fishery during the period of the 10-Year Management Plan. Total Atlantic Canada herring roe production increased from 400 t in 1983 to to almost 8000 t in 1990. During the last decade, Atlantic Canada fishermen supplied between 68 and 97% of herring roe used in Japanese production of flavored products. In 1988, approximately 72% of all purse seine landings in Scotia–Fundy were for roe extraction. Flavored herring roe provided a market for as much as 107 000 t of fish during the 1990 4WX fishery (estimated for roe production of 5356 t with a 5% yield).

Although the Japanese herring roe market has been dominated by the Atlantic Canada production, other countries have also responded to the development of this new market, and in recent years other sources of supply, notably Ireland and the Netherlands, have gradually been displacing the Canadian catch and contributing to an oversupply of roe in the Japanese market and a downward trend in prices.

Harvesting Sector

Harvesting of Atlantic herring has evolved over time according to changing market conditions, technological innovations, stock availability and management measures. The harvesting or primary sector of the herring fishery consists of three main gear types: gillnets, weirs and purse seines. The gillnet fleet is based in southwestern Nova Scotia, weirs are located predominantly on the New Brunswick side of the Bay of Fundy, and purse seiners are based in Nova Scotia and New Brunswick (Fig. 2).

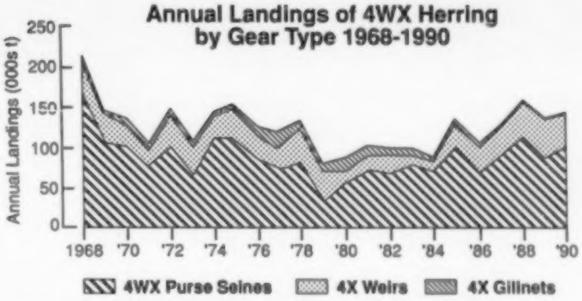

FIG. 4. Annual landings (mt) by major gear types in the 4WX herring fishery.

Gillnets

Gillnets have been used to take herring concentrated on or near spawning grounds since the middle of the 19th century. Nets are either set and tended or drift alongside the vessel in the strong tidal currents of the Bay of Fundy.

In the latter part of 19th century, a Grand Manan gillnet fleet of over 100 boats had peak landings of 10 000 t, but this fishery terminated by 1890 (Kearney 1983) due to collapse of the Grand Manan spring spawning stock. The southwestern Nova Scotia gillnetters numbered about 100 in 1952–63 with catches averaging 4500 t. Between 1976 and 1980 their number reached 250–300 vessels with annual catches of 10 500 t (Fig. 4).

Recent gillnet landings peaked in 1977 (in response to high herring prices), but have subsequently dropped to almost nothing as processors have moved more and more to purse-seine-caught fish. Buyers cite improved quality of fish and stable supply as their main reasons for this shift in source. Gillnetters, however, suggest that lower prices for higher volumes of fish from purse seines have resulted in the demise of the gillnet fishery (see also Isnor and Landers (1988) on the quality issue, and Kearney (1983) on gear conflicts and processors' preferences).

Gillnet drift net fishing effort concentrates on spawning aggregations for the lucrative herring roe market and for pickled herring and bait. Currently, daily catches average up to 10 t. Drift gillnetting employs two to four persons per vessel. Capital investment in gillnet vessels (multi-purpose boats and direct for other species like lobster, groundfish, swordfish or sharks) vary from $50 000 to $100 000 with approximately $10 000 attributable to the herring fishery.

Weirs

For well over a century, coastal weirs have been used to catch predominantly juvenile herring which aggregate near shore. Weirs are stationary traps consisting of stakes driven into the ocean bottom, connected and covered with brush and twine. The shapes vary, but basically consist of a heart-shaped trap and a leader (fence) extending from the weir to the shoreline.

In 1850, as many as 60 weirs were operational in the Passamaquoddy Region (Doucet 1960) and a similar number on the adjacent coast of Maine (Moore 1898). The increasing demand for canned sardines increased the number in Canadian waters (Charlotte County) to 507 in 1919 (Doucet 1960). These weirs supplied a growing Canadian sardine industry plus almost all excess demand for herring by American canneries. The proximity of this fishery to the USA has also given rise to a special fresh herring trade arrangement, for over a century now, whereby catches of each national zone can be landed in ports of either country without restrictions (Cook and McGaw 1988).

In the 1970's active weirs (on the New Brunswick side of the Bay of Fundy) were limited by license to about 240. Currently, over 200 active weirs are located along the shore and around islands of the western Bay of Fundy (west of Saint John) and fewer than 20 on the Nova Scotia shore of the Bay of Fundy, south of Digby. Catch rates vary depending on hydrographic conditions and migratory fish patterns. Apart from the restriction on weir numbers, there is no regulatory limit on the total weir catch. Juvenile herring taken in the New Brunswick fishery have been thought to originate in 5Y or 5Z and have been excluded from the 4WX TAC. In the same area as the weir fishery, small seines are used to enclose herring in coves or along the coast in the so-called "shutoff" fishery.

Weir fishing remains virtually unchanged since its inception. The Canadian (and to some degree the U.S.) sardine industry continues to be the major market for weir catches. The use of weirs is advantageous for the sardine market because of the general small size and good condition of fish caught and the proximity to processing plants. There is also an advantage over purse seines and gillnets in that "feedy" herring (herring with food in the gut) which would be undesirable for processing can be kept in weirs until their guts clear.

Weir landings have averaged 30 000 t per year during 1983–91 with a landed value of approximately CAN $6.5 million annually. Capital costs for weir construction vary, but average costs for poles and strung nets amount to approximately CAN $5000, with additional costs incurred for weir harvesting by seine.

The weir fishery has been characterized by interannual variability at individual sites. Landings in many weirs slumped in the early 1980's, and profitability has been marginal. Weir fishermen are concerned that developments in the burgeoning salmon aquaculture industry are displacing weirs and will disrupt the availability of herring in that the presence of salmon in cages may discourage herring schools from entering waters traditionally fished by weir operators (Stephenson 1990). Biological evidence suggests that the poor landings in the weir fishery are the result of changes in availability of juvenile herring (caused by behavioral patterns), rather than abundance. To compound problems, storms have been responsible for wide-scale damage to weirs and reduced production in some recent years. Government subsidies (for storm damage) and recent over-the-side sales of larger weir caught herring to foreign vessels have improved the economic position of weir operators.

Purse seines

Small purse seines were first used to remove fish from herring weirs. In 1934 the first purse seiner was used outside of a weir using oar-powered dories to catch mature herring. In following years, improvements in technology (Johnson 1966) included the introduction of powered seiner (1936), echo sounder (1946), diesel power (1948), sonar and radar (1950), fish pumps (1954), and power blocks (1955). In 1962, a hull form and hold for carrying herring was developed which allowed seiners to become self-contained fishing units, independent of carriers.

The purse seine fleet has dominated the fishery since the 1960's. The purse seine sector has taken as much as 175 000 t in 1968 (White and Mace 1988, p. 506), but in recent years (1984 to 1989) has taken an average of 90 000 t (nominal landings) in approximately 2000 sets per year (Power and Stephenson 1991), with an average landed value of CAN $13 million (1991). Numbers of vessels fishing Canada's East Coast, including 4WX, have been as high as 107 in 1968 (including a number transferred from the West Coast after collapse of that herring fishery), but with limited entry introduced in 1970 and declining herring meal markets, their numbers decreased to 55 by 1974.

Since 1983, the purse seine fleet of the Scotia–Fundy Region has been confined to fishing herring only in the waters of NAFO Div. 4WX comprising Chedabucto Bay, Scotian Shelf and the Bay of Fundy. The regional fleet was divided into three classes: Class "A" or "non-mobile" vessels, which before 1983 fished only in the Bay of Fundy; Class "B" or "mobile" vessels, which fished both the Bay of Fundy and Chedabucto Bay prior to 1983; and Class "C" being processor-owned vessels. Replacement costs are up to CAN $4 million (1991) for larger "mobile" vessels and upward of CAN $1 million for even the smaller "non-mobile" vessels. Purse seine vessels have crews of about six persons. Seining typically takes place at night with catches between 30 and 300 t. Landings are made within 12 h of capture (Power and Stephenson 1987, 1991).

Secondary Sector

Scotia–Fundy Region herring is currently processed and sold in a variety of food products including fresh and frozen round/dressed herring, fillets, butterfly fillets, cured, canned, smoked and roe. Herring is also used for the production of meal and oil as well as bait, and scales are used in the production of pearl essence. In 1991, there were 70 companies which sold or processed herring in the Scotia–Fundy Region employing at a peak of roe production approximately 2300 people. The sardine industry employs about 1800 people (300 full-time) and its production is valued at $70 million per year (Stephenson 1990).

The importance of various herring product forms has been relatively stable (in volume terms) for all of Atlantic Canada since 1955. Frozen fillets, pickled and vinegar cured and canned herring became more important with the elimination of the meal fishery (Fig. 5). Production of fresh fillets and frozen blocks have not played a major role in the Atlantic herring industry. Of interest has been the emergence and domination of the roe market, accompanied by an increase in the importance of meal and oil production and aquaculture feed which utilize carcasses, during the last 10 yr.

The short season and high volumes of herring processed during the roe fishery (July through October) in the Bay of Fundy have caused a problem of waste carcass utilization. Methods of carcass disposal have included further processing, dumping at sea, land filling and conversion to meal at fishmeal reduction plants. In 1987, for example, almost 73 000 t of carcasses were utilized as follows: 10% was pickled or filleted, 57% was reduced to meal, 15% ended up in landfills and 18% was dumped at sea (Canadian Fishery Consultants 1988). By 1992, there were six meal reduction plants in Nova Scotia and one in New Brunswick which together were capable of accommodating most peak periods. In 1991 there was no legal carcass dumping at sea and land filling has been forbidden since 1988. The problem has been reduced to some degree by the coincident emergence of a local demand for herring as salmon feed in an expanding aquaculture industry.

Management History

Pre-1965

The 4WX herring fishery developed gradually over about a century, from a subsistence fishery to a commercial fishery. Early management measures were directed at restricting herring harvesting behavior and effort for purposes of stock conservation. These included, for example, penalties for allowing fish to die in weirs, the government intervention to ban torching (attracting herring with light) (1884), closure of the summer spawning grounds to gillnets (1854), and prohibition of purse seining in open waters (1891). In 1903, the ban on purse seining was lifted by the Canadian federal government for harvesting Pacific salmon. By 1934, seiners on the east coast were fishing for herring in open waters.

Pre-Management Plan Period, 1966–83 (Table 1)

Expanding world markets and the apparent abundance of Atlantic herring resources led to a conference in 1966, sponsored by the Federal–Provincial Atlantic Fisheries Committee (Canada 1966) aimed at laying the groundwork for major expansion in the Canadian Atlantic herring fishery. This led to subsidized expansion of the herring fleet and of processing capacity, mainly for meal and oil products. When the Pacific herring fishery was closed for conservation purposes in 1968, 16 large purse seine vessels were transferred to the east coast to take part in the still expanding meal fishery (Canada 1991). However, by 1970 there were concerns for the state of herring stocks and the government responded by introducing limited entry for herring purse seiners and weirs.

Continuing signs of decline in resource abundance during the early 1970's led to further restrictions. On the impetus of the International Commission for the North Atlantic Fisheries (ICNAF), the Canadian government, in consultation with industry, adopted Canadian catch limits for herring in the Bay of Fundy and the Scotian Shelf regions (ICNAF subareas 4 and 5) for the 1972–73 season. At the same time, the United States agreed to ICNAF catch limits in subarea 5 (Gulf of Maine and Georges Bank). This was the first application of nationally allocated annual TACs in the management of a commercial fishery (May et al. 1981; Halliday and Pinhorn 1985; Iles 1993).

The imposition of annual herring TACs after 1972 seems to have induced a classical "race for fish" response among harvesters (see Doucet 1981), with increased competition among harvesters to procure larger portions of the total catch leading to increased investment in fishing capacity, and better equipped, larger vessels. In turn this resulted in shorter seasons (less time to catch the TAC due to the expanded fishing power), higher costs of fishing and processing, and a widening of the gap between low and high harvesting income earners. The market for herring meal was declining and the price of raw herring for reduction fell from about $60 per tonne in 1973 to less than $30 per tonne in 1975. It was also obvious that herring abundance was far below levels that maintained the high catches of the late 1960's. With allowable catch limits of 100 000 t, the 57 seiners operating in 1975 were barely managing, and the expectation that the situation would not improve in the coming years led many to believe that the fishery was on the verge of collapse.

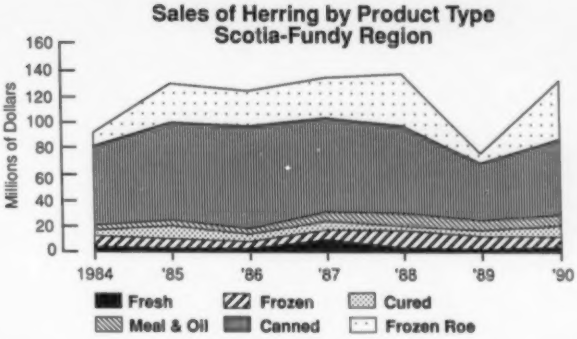

FIG. 5. Value (CAN$ millions, sales) of major herring products produced in Scotia–Fundy Region; 1984–90.

The 1972–75 period was also a period of self-organization of the herring fleets, with the formation of influential associations including the Bay of Fundy Purse Seiners (founded in 1972), the Atlantic Fishermen's Association (1974), and the Weirmen Association (1975). As a consequence of the disastrous period of low prices for meal, costly expansion, and declining stock abundance, purse seiners approached the government of Canada in May 1975 to seek income support assistance for that year (Kearney 1983, p. 138). This prompted a review of the East Coast herring fishery (Mitchell and Lennon 1975), which emphasized the need to improve the bargaining power of fishermen vis-à-vis raw fish buyers, and encouraged use of herring for food production as the means of increasing prices for raw herring paid to fishermen.

In November 1975 government biologists, managers, processors and fishermen met to implement a restructuring of the herring fishery. The mandate of the "Bay of Fundy Project" (Iles 1993) was to discuss the fishermen's request for subsidies for the poor 1975 season, and to examine the problems of maintaining longer term viability of the industry. The first task was to convince fishermen that they had the support of the Minister of Fisheries and Oceans who was serious in efforts to resolve the difficulties in the herring fishery, and to establish the potential size of the harvest in the short term. Through a series of meetings in the winter of 1975–76, the Project team, in consultation with fishermen's groups, assured fishermen that the requested deficiency payments would be forthcoming. The results of the herring abundance forecasts predicted a lower volume of herring harvest than in past years and reliance on the low price meal fishery would undoubtedly mean economic ruin to most harvesters. Accordingly, the Project team recognized the need to convert immediately to herring for food production. However, there was obvious concern that domestic processors would not be able to handle an approximate fivefold increase in the production of herring for food and an elimination of their meal reduction facilities already in place. It became necessary then to (1) extend the season to control the rate of supply of herring for food production and (2) support and subsidize the conversion, expansion and value loss of processing capacity for food.

To achieve an orderly disposition of the herring harvest, it was deemed essential to organize the harvesting component to act collectively to strengthen fishermen's bargaining power over the domestic marketing of raw fish, and to discourage individual harvesters from consenting to marketing arrangements with processors. The purse seine fleet, being the most affected of the

TABLE 1. Milestones of the purse seine period, 1966–83 (sources: Kearney 1983; Peacock and MacFarlane 1986; MacFarlane 1989; Iles 1993).

Period	Description of event
1966	Canadian federal government sponsors industry-wide conference to promote (and subsidize) expansion in herring plant and fleet capacity primarily for the meal fishery
1968	Pacific herring fishery is closed due to over-fishing; 16 purse seine vessels transfer to east coast to take part in expansion of meal fishery
1970	Canadian federal government imposes limited entry licensing for purse seiners
1972	ICNAF establishes national quotas for Atlantic herring; this is the first application of annual TACs by nation
1972	The Atlantic Herring Management Committee (AHMC; 1st fisheries advisory committee) comprises harvesters, processors, marketers, and biologists, economists, enforcement personnel and senior managers, first meets in June
1972	N.B. purse seine captains organize the Bay of Fundy Purse Seiners Association to lobby for greater access to U.S. stocks
1974	N.S. purse seiners (southwestern Nova Scotia) associate with Atlantic Fishermen's Association to influence government policy
1975	Grand Manan and Campobello Island weirmen organize the Fundy Weirmen Association mainly to lobby for a catch insurance scheme; purse seiners apply to government for deficiency payments; Minister sets up Inquiry; Bay of Fundy Project for restructuring of the herring fishery begins
1976	The "Club" is formed: Atlantic Herring Fishermen's Marketing Cooperative (AHFMC) comprised of seiners and a few gillnetters; the AHFMC negotiates herring prices with processors and directs vessels to plants; a system of suballocating the TAC among individual boats is established along with weekly boat quotas
1976	Canadian fisheries minister LeBlanc invokes Section 29 of the Act and bans the use of whole fish for meal; subsidies are awarded to processors to convert and expand facilities to food fishery; over-the-side sales (OSS) are authorized to Poland to provide alternative markets and to increase domestic prices; deficiency payments are made to purse seiners for poor 1975 season
1977	4X Herring Working Group established providing links from DFO to Atlantic Herring Mannagement Committee
1979	Processors agree to meet Canadian government's conditions for discontinuing OSS; no export licenses awarded to AHFMC for OSS contracts
1979	Argyle-Barrington purse seiners split from AHFMC over marketing arrangements and unite under South-West Seiners (SWS); each captain makes own marketing arrangements (unlike AHFMC, SWS is not a marketing cooperative)
1980	Incidences of "red feed," low prices, small herring, and Gulf vessels in the Bay of Fundy lead to dissatisfaction with AHFMC; under-reporting of catches becomes commonplace; in an attempt to recoup support, AHFMC and SWS take legal action against DFO for loss of potential herring catches amid pessimistic stock projections and low TACs in previous years
1981	In spite of increased TAC over previous years, economic conditions deteriorate; OSS reinstated despite processors' objections (deal with Poland falls through, Swedish intermediary sets up contract of direct sale to USSR)
1981	Fishermen realize they are not legally restricted from exceeding AHFMC boat quotas; DFO initiates action to legalize boat quotas; Atlantic Coast Herring Regulations amended accordingly
1981	Fisheries Minister LeBlanc announces that there is no place for marketing arrangements in the herring fishery; government prepares to abandon AHMC for 3 regional advisory committees and to establish boat quotas for all Atlantic purse seine herring fisheries
1982	Fundy Coordinators formed to market herring collectively from AHFMC, SWS, and ASG (Gulf)
1983	Task Force on Atlantic Fisheries final report is released; recommendations include: vessel quotas for seiners, 5-yr buy-back program funded by industry levies, and stringent measures for misreporting
1983	10-Year Management Plan inaugurated; industry consultations via Herring Advisory Committees; license moratorium; separation of fleets (Scotia–Fundy vs Gulf), mobile and non-mobile components; single area management 4WX; vessel quotas; fleet reduction program through quota transfer restrictions

harvesting groups, and having already organized themselves, became the focus of the Project team's attention. The major objective of achieving economic viability for the fleet led "immediately and inevitably to a boat sub-allocation scheme" (Doucet 1981, p. 12). Individual boat allocations were negotiated that gave vessels the opportunity for economic survival in the upcoming 1976 season, and at the same time ensured an adequate stock.

Early in the spring of 1976, the Atlantic Herring Management Committee (AHMC) was presented with the allocation scheme developed among the purse seiners. The AHMC, responsible for final advice to the Minister, was composed of representatives of fishermen, processors, and government staff members. In the end, and despite objections by processors to the proposed elimination of meal production, the committee accepted the proposal for the 1976 season ". . .both because of its inherent reasonableness and the offer of improved market opportunities and because of the fairness of the reallocation schedule" (Doucet 1981, p. 14). In April 1976, the Minister of Fisheries announced a program of $750 000 in deficiency payments to purse seine fishermen of the Bay of Fundy as part of a larger set of reforms that would be initiated in the herring fishery. As a direct result of consultations with the industry by the Bay of Fundy Project, a number of sweeping reforms were announced for the 1976 season. These included:

(1) Invoking Section 29 of the Fisheries Act that banned the use of whole herring for meal.

(2) Financial assistance programs to herring processors for the conversion and expansion of facilities for food herring production.

(3) A system of suballocating the total fleet quota to individual vessels including limits on weekly catches by each vessel. Fishing by groups of fishermen was encouraged primarily to avoid dumping and discarding. Participating fishermen were permitted to pool their weekly vessel quotas.

(4) The formation of the Atlantic Herring Fishermen's Marketing Cooperative (AHFMC) ("the Club") whose function it was to negotiate herring prices through collective bargaining on behalf of its member harvesters with processors and, by radio dispatch, direct seiners to various plants to land their catches. The AHFMC also took on the responsibility of administering and monitoring the individual vessel quotas. This was managed through a system of log records and delivery slips.

(5) The authorization of direct sales "over-the-side" (OSS) of herring to Polish factory vessels. The OSS program was conceived as a stop-gap measure to aid herring harvesters while processors were converting capacity to food production, and to stimulate higher prices in the domestic market. As an incentive to join the AHFMC, OSS sales were restricted to members of the AHFMC only.

Initially, only 27 of the 53 purse seiners agreed to join the AHFMC, although all had agreed to vessel allocations. However, the operation of the self-imposed and self-managed allocation system was successful (in spite of a history of 30% and higher under-reporting annually, the 1976 quota of 61 700 t was not reached), the average prices for herring paid to AHFMC members versus nonmembers improved ($80 per tonne versus $51 per tonne, the difference due in large part to the $105 per tonne for OSS and the AHFMC negotiated price of $75 per tonne for sale to domestic processors), and only 34% of harvest was for meal in 1976 versus 80% the previous year. The 1976 season lasted 6 wk longer than the poor 1975 season and, although total catch was down from 84 000 t in 1975 to 60 000 t in 1976, total landed value had increased from CAN $2.8 million to CAN $4 million (Doucet 1981, p. 15–16). By the beginning of the 1977 season, nearly all seiners had joined the AHFMC.

The system of individual vessel quotas, weekly boat limits, and the log and delivery slip monitoring system proposed under the Bay of Fundy Project and adopted by the AHFMC were not legally binding regulations imposed on the harvesters. Rather, they were self-imposed, informal arrangements agreed to collectively by the purse seine captains and administered by the AHFMC. As such, they represent the first application of an individual vessel quota scheme in a large commercial fishery. It is even more remarkable that this system was one of the first fisheries to be managed in large part by the fishermen themselves with minimum government involvement (Doucet 1981, Iles 1993). The individual vessel quota scheme formed the basis for the 1983 10-Year Management Plan for herring, and ITQs have become a common element of fisheries management programs in Canada and around the world in the 1990's.

The situation in the herring fishery continued to improve in 1977 and 1978. The allocation system of 1976 was continued with minor changes to the vessel allocation scheme. The conversion of the meal to the higher valued food fishery (and the accompanying changes to harvesting and processing quality control especially in refrigeration), rising prices in the European market amid the collapsing North Sea stocks, and the optimism created by the success of the new marketing regime injected new buoyancy in the fishery. Prices to seiners increased to $104.84 per tonne in 1977, to $187.50 per tonne in 1978, and to $250 per tonne in 1979 (Kearney 1983, p. 158).

In 1979, marketing difficulties occurred when catches of the main herring year-classes that supported the food fishery diminished and processors could not meet margins due to the large proportion of small fish. As a result, less than half of the seiner TAC of 59 000 t was landed. As well, there developed disagreements within the AHFMC over the regional distribution of shore facilities, access rights to traditional fishing grounds, and catch allocations (White and Mace 1988). Ultimately, the monopoly of the AHFMC was eroded when 13 purse seiners broke off to form their own marketing arrangements with processors. The seiners were all part of the South West Seiners group originally formed in 1976 as a holding company to manage the purchase of nine vessels formerly owned by a large fish meal firm. These tended to be large vessels and their separation from the AHFMC significantly reduced its role in determining the amount, location, and prices of harvested herring. Moreover, it induced other harvesters within the AHFMC to consider marketing arrangements with processors. Similarly, herring gillnetters left the AHFMC in 1979 and formed their own committee within the Nova Scotia Fishermen's Association. This same year herring processors successfully lobbied the government to discontinue the OSS program and no contracts were permitted during 1979 and 1980.

The low catches of the 1979 season led the government to restrict quotas in 1980. The situation worsened as a result of high fat content in herring throughout the year and significant problems of "red feed" in the gut which made the fish difficult to process. These problems and the erosion of the AHFMC led to a breakdown of the monitoring system and many incidences of under-reporting by the disgruntled seiners. By 1981, however, poor prices and markets forced the government to reinstate the OSS program.

In 1981, amidst rampant under-reporting of herring catches and the legally non-binding AHFMC quota system, the government of Canada moved to amend the Atlantic Coast Herring Regulations and legalize boat quotas. At the same time it established new regional advisory committees (including the Scotia–Fundy Herring Advisory Committee) to coordinate and continue the vessel quota system for purse seiners in a consultative setting with industry (Kearney 1983, p. 330).

Early in 1983, the Task Force on the Atlantic Fisheries was released (Kirby 1982). Its recommendations for the herring fishery included the continuance of vessel quotas, a rationalization of the purse seine fleet through a buy-back program funded by industry levies, and stringent penalties for catch misreporting. The Task Force recommendations and the historical developments in the fishery to this point provide the background for the 10-Year Management Plan for 4WX herring initiated in 1983.

The 10-Year (1983) Management Plan (Table 2)

Beginning in 1981, through the workings of the newly formed

TABLE 2. Year-by-year synopsis of the 1983 Management Plan period (sources: Stephenson et al. 1986, 1987, 1990, 1991; Stephenson and Power 1988, 1989; MacFarlane 1989; Nowak 1992).

Period	Description of event
1983	Scotia–Fundy Herring Advisory Committee (SFHAC) replaces AHMC; annual management plans set each fall by consensus building among industry and DFO; plans recognize: distinct fall seine fishery off N.B. coast, winter fishery for adults off N.B., Nov.–March fishery for overwintering adults in Chedabucto Bay, allowances for N.S. weirs, gillnets, traps, no quotas or allowances for N.B. fixed gear, quota for midwater trawler
1984	Emergence of the Japanese herring roe market
1985	Misreporting is considered a major issue and catch records for 1973–84 are revised at 1.15 to 1.77 times those reported. SFHAC elicits cooperation of seiners under the Management Plan to provide accurate catch and fishing data; systems of verbal hails, delivery notes, purchase slips, ship logs, and weekly license renewals are established; TAC is increased by 56% over 1984 and exceeds biological advice, but is linked to increased monitoring; 185 written reports of violations are made; regulatory package for increased monitoring and enforcement drafted; revision and approval processes are slow
1986	To maximize use of quota purse seiners are given more than guaranteed 80%, and inshore gear are given an allocation less than 20% based on expected maximum performance (market capacity). Increased prices over 1983 level, higher TACs, roe market dominates
1987	Earnings of seiners double over 1983 levels due to roe market, increased prices, and higher TACs. Limited spawning closure (3 d/wk during peak spawning) implemented to protect Trinity Ledge spawning component
1988	Higher TAC (151 200 t) with purse seine sector allocated 88%. Experimental roe fishery allowed in Scots Bay (upper Bay of Fundy) which has been closed for several years; opening of fishery determined by roe yield from test vessels results in high yield. Trinity Ledge closed for 3–4 d/wk during spawning
1989	TAC remains at 151 200 t. Price dispute delays and reduces roe fishery. Redrafted monitoring and enforcement regulations appear in the *Canada Gazette Part II* (April 26). Upper Bay roe fishery allowed, but did not take place due to price dispute. Trinity Ledge closed for 18 d during spawning
1990	Purse seiners allocated 94% of 151 200 t TAC. Misreporting documented in the summer fishery precludes analytical assessment
1991	New monitoring and enforcement regulations including fish hold volume calibration and measurement introduced to the Fall fishery in Chedabucto Bay
1993	10-Year Management Plan scheduled to end

Scotia–Fundy Herring Fishery Advisory Committee, DFO consulted extensively with the industry in the formulation of a management plan for the herring purse seine fleet in Atlantic Canada (Iles 1993). Consultations were held with seiner associations, operators and herring processors throughout Atlantic Canada. These consultations ultimately led to the formulation of 10-yr plans for the management of herring purse seiners of Atlantic Canada introduced in 1983. The primary objectives of the Scotia–Fundy Region Plan included (i) fleet rationalization, (ii) restoration and conservation of the herring stock, and (iii) improved economic stability for the fishery. Specifically, the Plan instituted the following regulatory measures: (1) separation of the Gulf of St. Lawrence and Scotia–Fundy fleets via sector management, (2) saleable individual vessel quotas, (3) guaranteed vessel quotas for 10 yr based on annually determined TACs, and (4) a moratorium on the issuance of new licenses (Canada 1991), as follows:

(1) *Sector management.* The separation of the purse seine fleet was based on the recognition of the discreteness of Bay of Fundy and Gulf of St. Lawrence herring stocks. This separation of the fleet to the exploitation of distinct herring stocks allowed for discrete management of the two stock complexes fished by different purse seine fleets. It was recognized, however, that the Sydney Bight (4Vn) area was one of stock mixing, making a separate stock assessment for the area impossible, and proper allocation of catches difficult (Stephenson and Power 1990).

In separating the two fleets, there was a problem created by the inequity in terms of tons of herring available per seiner using the strict definition of regional boundaries. In order to compensate, the Gulf fleet was given access to the northern portion of the 4Vn area to fish intermixed stocks. Tagging results indicated that herring north of Point Aconi, Cape Breton, were predominantly of 4T origin and that the 4WX stock predominated south of this line. Despite scientific concerns for fishing in this area where stock definition is complicated by mixing, and Regional understanding that the access was to be temporary, a fishery of 3000–5000 t annually has been allowed in recent years.

(2) *Individual transferable quotas (ITQs).* The key management measure designed to allow reduction of the numbers of active purse seiners was the system of transferable vessel quotas. While the fishery had been managed by boat quotas since the 1976–77 season, the vessel allocation procedure was in dispute and the rights to allocations were not transferable. Under the Plan, the initial allocation scheme recognized different segments of the fleet based on size and historical involvement (Crouter 1985). The 49 vessels of the Scotia–Fundy fleet were divided into "mobile" vessels which were predominately larger and historically fished outside the Bay of Fundy (primarily in Chedabucto Bay) for part of the year, and "non-mobile" vessels which consisted the Bay of Fundy fleet of smaller, mostly wooden seiners who had no history of fishing outside the Bay of Fundy. The existing 20 licensed mobile vessels were each awarded 2.7% of the overall 4WX purse seine quota. The 29 non-mobile vessels were each offered 1.6%.

The allocation system also encouraged fishermen ownership rather than processor ownership of vessels and licences. While fishermen-owned vessels were permitted to accumulate quota from other fishermen and from processors, processors were not permitted to gain quota from fishermen-owned vessels. This effectively prevented processors from increasing their share of vessel quotas. Moreover, to guard against concentration of quota in a few vessels, an upper limit of 4% was placed on the amount of quota which could be accumulated by any one vessel.

Finally, restrictions were also placed on the transfer of vessel quotas under the Plan. Since a primary objective of the Plan was fleet reduction and not to encourage speculation in the sale of quotas, any vessel owner who wanted to sell quota was required to sell the whole quota and then leave the fishery.

(3) *Guaranteed quotas for 10 yr.* As part of the Plan's objective to achieve "stability" in the fishery, participating purse seiners were guaranteed quota privileges (unless otherwise transferred) over 10 seasons beginning in 1983. This included a guarantee that the purse seine fleet would receive 80% of the annual TACs for the Scotia–Fundy herring fishery. In return, quota holders were expected to take part in the annual harvest of herring and to capture their share of the TAC set annually in the fishery. All pooling arrangements including rotational fishing among vessels or temporary buying or transferring of quotas were prohibited. The 10-yr period was instituted also to "ensure sufficient time for an orderly process of fleet rationalization to occur" (Canada 1991, p. 16).

(4) *Moratorium on new licenses.* Under the Plan, no new licenses for 4WX herring would be issued for 10 yr to purse seiners outside the Plan. Accordingly, changes in licenses could only occur among those participating originally within the Plan.

Annual Management under the Plan

The development of the annual 4WX Herring Management Plans under the 10-Year Plan was undertaken by the Scotia–Fundy Herring Advisory Committee (SFHAC), the successor to the Atlantic Herring Management Committee, Canada's first fisheries advisory committee that first met in June 1972. As its predecessor, the SFHAC has consisted of members of industry and DFO representatives (Table 3); however, the industry has become more fragmented, resulting in a larger and larger forum for debate within the committee.

The SFHAC has typically met twice a year (in the spring and fall) since 1983 to develop and implement the annual herring management plan for the region. Special meetings of working groups comprising all or a part of the SFHAC have been held to deal with specific aspects of the plan.

At its meetings, the Committee has heard the scientific advice (from CAFSAC and biological background to that advice), as well as any available regulatory, economic or other advice pertinent to the fishery, after which the Committee has attempted to reach consensus on recommendations for a draft TAC and operational plan for ministerial approval. Although the distribution of quota among the seiner fleet is set under the terms of the 10-Year Plan, the Committee has discussed various control measures, including the temporal and spatial guidelines for the fishery, the closure of spawning grounds on Trinity Ledge and in Scots Bay, the conduct of the over-the-side sales programs and the resolution of inter-sector problems in the fishery. The development of industry consensus has led to subsequent ministerial approval of the annual plans.

The annual 4WX Herring Management Plans have incorporated the fundamental principles of the 10-Year Plan for the management of herring purse seiners. These have included the consistent assignment of the distribution of annual quota to vessels, and for industry-financed fleet reduction through quota transfers. Annual management plans have also recognized: a distinct fall seine fishery along the coast of New Brunswick; a winter fishery for herring along the coast of New Brunswick; a November to March fishery for overwintering adults in Chedabucto Bay; a separate boat quota for a single midwater trawl; the recognition of an allowance rather than a quota for the gillnets, traps and weirs of Nova Scotia; no quotas or allowances for the fixed gear of New Brunswick since the juvenile fish taken in this area are more likely associated with the coastal stock of New England than with the 4WX stock; OSS markets as leverage against weak domestic and other markets for herring products especially when resource availability remains relatively high; the allowance of an individual boat quota within the annual TAC for purse seiners; and prohibition of quota pooling arrangements.

TABLE 3. Membership of the Scotia-Fundy Herring Advisory Committee (1991).

Harvester representatives
- Atlantic Herring Fishermen's Marketing Co-op
- Southwest Seiners
- Fundy Weir Fishermen's Association
- Grand Manan Fishermen's Association
- Eastern Fishermen's Federation
- Maritime Fishermen's Union
- Cape Breton Fishermen's Association
- Eastern Shore Fishermen's Protective Association

Processor representatives
- Comeau Seafoods Ltd.
- Connors Brothers Ltd.
- Continental Seafoods
- Independent Seafood Producers Association of Nova Scotia
- Lawrence Sweeney Fisheries
- Mirimichi Marine Products
- New Brunswick Fish Packer's Association
- Sable Fish Packers
- Schooner Seafoods
- Seafood Producers Association of Nova Scotia

Government representatives
- Canada Dept. of Fisheries and Oceans
- New Brunswick Dept. of Fisheries and Aquaculture
- Nova Scotia Dept. of Fisheries

In an attempt to spread fishing effort among all stock components contributing to the herring fishery, the annual management plan has also encouraged: intermittent closures of Trinity Ledge (a spawning area near the port of Yarmouth which has been subjected to disproportionately high effort, and has been the site of purse seine/gillnet gear conflict); the cautious development of a purse seine roe fishery in Scots Bay including a test fishery; and occasional experimental trips to offshore banks (e.g., Emerald, Browns, and Georges banks).

Table 2 presents a year by year synopsis of the major events of the post Plan period, 1983 to 1991, the last year for which data are available.

Analysis and Evaluation

Critique of the 10-Year Plan

The 10-Year Plan of 1983 had three stated objectives: (i) fleet rationalization, (ii) restoration and conservation of the herring stock, and (iii) improved economic stability of the industry. These were to have been achieved in part through the individual vessel transferable quota system which would allow fleet rationalization to permit viability of remaining vessels. We have examined the performance of the plan through critical evaluation of (a) the ability of the Plan to achieve its stated objectives, and (b) the appropriateness of these same objectives.

(i) *Fleet rationalization.* The Task Force on Atlantic Fisheries had concluded that the herring resources on the Atlantic coast could not support the purse seine fleet that was in existence at that time (1982), and specified vessel reduction goals of "some 20 to 25 vessels" (Kirby 1982, p. 125) for the 63 vessels in the combined Bay of Fundy and Gulf of St. Lawrence fleets. The Scotia–Fundy fleet was recognized as having excess capacity, as well as having a number of old vessels which were in need of improvements (particularly cooling systems) to be able to land fish of competitive quality (Peacock and MacFarlane 1986). However, the specifications of an "ideal" fleet size for the Scotia–Fundy fleet, or the desirable number of vessel reductions under the 10-Year Plan were not made explicit. The move to sector management effectively rationalized the fleet harvesting 4WX herring from 63 to 49 vessels. One can surmise from the percentage allocations limits of 4% instituted in the Scotia–Fundy vessel quota system of the Plan that the desirable long run evolution of this fleet would leave some 25 vessels remaining but the rationale for the 4% quota cap, or the minimum fleet size of 25 vessels, is not clear. An interim evaluation of the Plan (MacFarlane 1989, p. 47) summarized the logic:

> "There were 49 vessels in the Scotia–Fundy herring purse seine fleet in 1983. Half that number of vessels and a doubling of the quota per vessel would bring a measure of viability. An even smaller fleet would have been sufficient to take the catch if efficiency were the only objective."

In these terms, the observed consolidation of the Scotia– Fundy fleet from 49 seiners in 1983 to its current size of 40 seiners can hardly be viewed as having accomplished the implicit fleet reduction objectives under the Plan. Moreover, there has been a feeling within segments of the industry that the anticipated degree of fleet reduction was unrealistic and unwarranted, and the impression that any vessels which survived the 10-Year Plan would be considered viable and treated equitably in future plans.

(ii) *Restoration and conservation of the herring stock.* The long-term outlook at the beginning of the plan was of a stock that would allow a harvest of 100 000 t per year (Canada 1981, 1985). A correction of the historical catch record in 1985 to account for misreporting in the years 1978–84 (Mace 1985), combined with evidence of stock increase during the 1980's led to a changed view of the resource and the outlook was increased to approximately 125 000 t per year (Rivard et al. 1988). The improvement in stock status led to increased TACs and, problems of misreporting notwithstanding, the TACs that have been set since 1988, and perhaps as early as 1985, have not been restrictive. There is no doubt that limited entry and annual total catch limits in effect since the 1970's have contributed to the conservation of the 4WX herring stock, and this is in striking contrast to the fate of the neighbouring Georges Bank stock (which collapsed in the late 1970's (Stephenson and Kornfield 1990; Melvin et al. 1991)). The continuation of annual management principles under the Plan, and initiatives such as the Trinity Ledge spawning closure during the Plan, have maintained stock conservation. The Plan itself, however, has not directly contributed new management measures toward achieving the stock restoration and conservation objectives.

(iii) *Improved economic stability of the industry.* The separation of the Scotia–Fundy and Gulf fleets and guarantee of 80% of the Scotia–Fundy TAC for purse seiners improved the economic position and stability of these seiners, ensuring them a predictable share of the total catch. Furthermore, the increase in TAC and demand for Atlantic herring destined for the Japanese roe market since 1984 greatly buoyed the economic position of vessels participating in the herring roe fishery. High prices in this market probably improved the lot of many seiners to the point that economic viability was sustained, where it may not have been otherwise, thus calling into question the explicit goal of fleet reduction. The availability of this market, together with the under-reporting which occurred under the quota system, continued to support even marginal operators through the period of the Plan since 1984.

In summary, the 10-Year Plan has, in some respects, been successful – but not as planned in 1983 and, to a large degree, in spite of its specific objectives and institutionalized management framework. The viability of the fleet has improved due to the adaptability of the industry to changing markets, and herring abundance has not been adversely affected. It is not correct to associate this economic improvement with increased stability however, for the stability of the purse seine fleet is uncertain (Nowak 1992). The recent downturn of the Japanese roe market, outside competition, and a fall in roe prices threaten to return the fishery to the economic crisis position of the early 1980's.

The Plan, however, has not brought about the degree of consolidation of the fleet anticipated by the fleet reduction objective. This result is attributed to the inappropriateness of an inexplicit fleet reduction objective within the transferable vessel quota management framework. Hypothetically, transferable quotas should allow the industry to adapt itself to changing dynamics of markets, stock availability, processing capacity, etc., when unencumbered by rigid management regulations. Accordingly, inasmuch as the uncertainty of future events combine to dictate the design of appropriate and efficient fleet configurations, there is no need to set fleet targets. It is myopic to presume that a fleet configuration judged to be "best" for 1983 should also be appropriate over a 10-yr period.

On the one hand then, in that the Plan specified a fleet reduction goal, it can be criticized as not having achieved it. On the other hand, and more appropriately, the Plan is criticized for stating fleet reduction as a goal in conjunction with the adoption of transferable quotas. In fact, the Plan may be deemed successful in that it did permit industry to evolve within its quota system according to the changing circumstances that have occurred since 1983. The fact that it was not to the degree or in the manner anticipated by the Plan is the result of flaws in the specific form of the system of transferable quotas institutionalized in 1983.

Critique of the ITQ System

In 1983, it was understood that fleet rationalization to eliminate the existing excess capacity would come about as a consequence of industry-induced vessel trading through the transferable vessel quota system. As pointed out elsewhere for this fishery (Campbell 1981; Doucet 1981; Peacock and MacFarlane 1986), the quota system could not realize its positive aspects without proper monitoring and enforcement of catch by vessels, since individual quota systems reduce the need for "input controls" (restrictions on vessel size, gear characteristics, etc.,) but increase the requirement for effective "output controls." While misreporting was certainly recognized as a serious problem in this fishery long before the inauguration of the Plan in 1983, difficulties and delays experienced in installing a planned package of revamped regulations scuttled the effective operation of quota transfers and, along with it, the means of potential fleet reduction. There is evidence (MacFarlane 1989; Nowak 1992) that, in spite of the roe markets and improved stock availability over the 10-yr period, many vessels could not have maintained economically viable operations solely on the basis of their individual vessel allocations.

Secondary to misreporting and weak monitoring and enforcement, potential fleet reduction was also hampered by the specifics of the vessel quota scheme. The adoption of individual vessel quotas for the herring fishery was an attempt to derive the positive, and primarily economic, gains from this quasi-property rights management system. As Peacock and MacFarlane (1986) report, a major attraction of property rights schemes was that they offered a means of potentially avoiding problems experienced under other management regimes. These problems included excess capacity, rent dissipation, low incomes, overfishing, and stock depletion that the industry was experiencing up to the time the plan began. Moreover, this economic-based crisis of the herring fleet in the early 1980's (that was also being felt in other major commercial fisheries at this time (Kirby 1982)) prepared the way for major shifts in management approaches. There were a number of positive reasons for the adoption of individual vessel quota systems that were implicit in the 10-Year Plan (Copes 1986; Muse and Schelle 1989). These include the following potential benefits:

(a) *Economic efficiency*. Quotas promised overall improved economic efficiency that would come about from the greater control harvesters would have over catch. The quality of the catch would improve, while operating at lower fishing effort levels and costs rationalized to the size of the individual quota. Similarly, the fishing season would be lengthened since fishermen would be guaranteed their individual quotas thereby eliminating the "race for fish" syndrome typical of non-property rights management. In short, the quota system would permit harvesters to rationalize their operations toward maximizing their individual benefits from catching the assigned quota.

(b) *Rationalization through ownership and transfers*. Quota system characteristics of ownership (and thus intrinsic value) and transferability should have promoted overall rationalization of the harvesting activity. Differences in perceived value of owning quota (versus owning other economic goods of comparable value) would lead to transfers of lower-valued quota shares to higher-valued ones. The results would lead to a reduced and more economically efficient harvesting sector.

(c) *Stability in integrated operations*. Increased control of stock removals by harvesters also provided a means of smoothing out peak period catch and processing problems. Integrated operations would benefit from increased stability, improved quality and markets, and harvesters would benefit from higher prices and reduced competition on the fishing grounds.

(d) *Equity*. Finally, the initial allocation of quotas among fishermen presented the opportunity to introduce "fair" intermarginal and across-gear access to herring. It was deemed that equal shares within separate fleet components and the restrictions of quota limits would potentially lead to an equitable distribution of benefits to all harvesters.

While the Plan recognized the economic-based problems experienced by the herring purse seine fleet, it implicitly assumed that the benefits of individual quotas would be realized by adopting the system and allowing the industry the capability to configure itself through vessel transfers in a more economically efficient manner. However, partly due to the recent history of the fishery prior to 1983, the specific kind of quota system that was adopted, and lack of attention paid to problems associated with individual quotas, the expected benefits were not fully realized. There are a number of reasons why the quota system failed to achieve its potential benefits in this case. Chief among these was the misreporting of catches (under-reporting) which is a common problem associated with implementation of quota and quasi-property rights schemes (Copes 1986; Muse and Schelle 1989). The following discusses problems related to the effective implementation of quotas in this herring fishery.

(a) *Monitoring and enforcement*. Under-reporting of catches was "rampant" before the start-up of the Plan. Implicit in an Individual Quota management strategy was a reduction in the need for "input" controls (vessel and gear characteristics, etc.), but a critical requirement for adequate "output" controls (i.e., quota monitoring). By not resolving this in the operation of the Plan, the potential benefits of quota management were effectively scuttled. Ineffective monitoring and enforcement continued to plague this fishery. Improvements made in 1985 appear to have been short lived, and another reconstruction of the catch record was required recently (Stephenson et al. 1991, 1992). An effective integrated paper trail of catch reporting (Clark et al. 1989) did not exist for herring (until recently, fishermen were not required to determine the weight of catches; statistics were derived from records of delivery (delivery notes) to processing facilities), and there has been a lack of a legal framework to prosecute noncompliance, and corresponding difficulties on the part of the courts to uphold prosecutions.

(b) *Overcapacity*. Initial vessel allocations in 1983 were well below the harvesting capacity and traditional landings of many purse seiners, and were not perceived, by some, as having been fair. This being said, many vessels had an economic incentive to underreport in order to maintain viability — a conflicting goal alluded to in the Plan. As long as all harvesters were acting to maintain their own positions, there were no incentives to transfer quotas since increased catch could be taken by under-reporting. Accordingly, the value of purchasing quota was effectively reduced to the point that it added nothing to the wealth of the harvesting enterprise.

(c) *Restrictions on transferability*. The degree of transferability is thought to be important to the success of ITQ systems; it is generally considered that quotas should be completely transferable (Pearse 1991). The "all-and-exit" and 4% maximum restrictions on quota transfers under the Plan effectively reduced the potential for trades and provided no incentive to reduce discards or under-reporting of catches. Indeed, as Peacock and MacFarlane(1986) report, of the seven vessels that transferred quotas in 1983, four of those had gone out of business prior to sale of the quota, one processor consolidated two vessel quotas into one quota, and only two independently owned and operated seiners actually sold out. There have been only two quota transfers in later years of the Plan, although a number of vessels have been replaced. The under-reporting problem notwithstanding, a more divisible transfer system with in-season transfers would attribute more real value to owned quotas and allow the potential for efficient trades.

(d) *Equity in allocations*. Initial vessel allocations differentiated between sizes of vessels with corresponding differences in relative costs. The Task force on Atlantic Fisheries (Kirby 1982) imagined movement toward an efficient distribution of fishing rights in the long term in an "ideal" fleet "comprising vessels in the 65- to 75-ft range equipped with refrigerated seawater or equivalent features to ensure quality landings". This was to be achieved "fairly quickly" through "equitable or fair initial allocation" on the basis of "reported relative catches over the past three years." However, the performance of the existing fleet suggests that large economic and behavioral differences exist among fishermen even within the same size or class of vessel. The imposition of a strictly uniform system effectively removes incentives for highly efficient operators, or "highliners" to exist. In the absence of strong enforcement, vessels have been able to maintain additional margins through under-reporting or special arrangements with processors.

(e) *Herring markets*. This fishery has been highly dependent on the whims and changes in the market. On the positive side, the ability to adapt to these changes has been a characteristic of the herring fishery. During the period of the Plan, the lucrative Japanese roe fishery dominated the catch of herring. However, this also resulted in an intense fishery on pre-spawning herring during a short season. Consequently, potential stability in integrated harvesting and processing operations were nullified as vessels and processors sought to cash-in on this market. It became another "race for fish" (Copes 1986). Under-reporting of catches for roe and over-capitalization and use of carrier vessels are also symptomatic of this situation.

(f) *Quota imposition*. The initial quota allocation under the 1983 Plan, unlike the voluntary agreements of the 1976 industry initiated quotas, was imposed on fishermen (albeit after consultation (Canada 1981)). Dissatisfaction with this imposition may have contributed to under-reporting. It was recognized early in the Plan that without a clear indication of the eventuality of quota ownership at the end of the 10-yr period, there may be a tendency for participants to "stand pat" and not trade their quotas until the future management process and fishing rights allocations are declared (D.A. MacFarlane 1984, DFO, personal communication). This seems to have been the case, and several vessels have been riding out the plan hoping for redistribution of quota in a new plan.

(g) *Resource changes*. Herring abundance increased over the period of the Plan. The effect of this positive biological variability led to more flexibility on the part of the SFHAC and government to allow higher catches and annual TACs during the Plan period. This in turn led to the continuance of otherwise marginal harvesters in the fishery. Paradoxically, the improved status of the resource has had the opposite effect to achieving the Plan's fleet reduction objective by maintaining minimal returns to existing harvesters through increased availability.

(h) *Plan duration*. Sources of variability in all areas (e.g., markets, stock abundance, etc.,) also conspired in the long run to diminish the success of the Plan. In retrospect, the 10-yr horizon of the Plan was essentially designed in a static context in reaction to the existing (early 1980's) crisis situation. It was not designed to be flexible in the face of a wide range of possible scenarios that could and did eventually come to pass in this fishery during the Plan period. On the other hand, it was too short to provide the feeling of ownership — and some considered it a temporary arrangement. What was required was a realistic long-term strategy combined with a framework for making decisions on short- and long-term management alternatives within the Plan that could take advantage of the highly variable, dynamic system, without compromising long-term goals.

(i) *Plan scope, review, and structure*. The 10-Year Plan was applied as a series of annual plans. Almost all issues were open for discussion during annual plan formation with the result that the SFHAC made decisions and took action which in retrospect may have been inconsistent with the original intent of the plan (allowing quota pooling arrangements, changes in restrictions on use of "mobile vessel" quota, maximizing seiner quota by placing inshore gear on allowance, lease and shared arrangements, etc.). While these may have all been justifiable actions and may have represented appropriate short-term strategies – they were inconsistent with the original intent of the Plan.

Conclusions

The Scotia–Fundy 10-Year Plan was developed during a time of turmoil in the herring fishery. Problems including overcapacity, reduced stock size, depressed markets and overfishing indicated that the resource could no longer support the major purse seine sector as it was then structured (Kirby 1982; Peacock and MacFarlane 1986). The Plan attempted to rationalize (reduce) the purse seine fleet, restore and conserve the stock, and stabilize the fishery economically through a long-term arrangement involving restricted entry (sector management and limited entry), a guaranteed fleet allocation (80% of TAC), and individual transferable vessel quotas.

The 4WX herring fishery is one of the few large herring fisheries world-wide that has not "crashed" due to changes in market demand or stock collapse. Early catch restrictions (limited entry since 1970, annual TACs since 1972) and restrictive markets for herring have helped in meeting stock conservation goals. Individual quotas, on the other hand, have probably not added anything new to stock conservation (Muse and Schelle 1989, p. 109; MacGillivray 1990, p. 8), in that unchecked misreporting could have compromised stock status. Economic stability and viability in the commercial sector has been maintained artificially due to misreporting. If restricted to the quota, several vessels would not have been viable.

Recent years have seen reasonable (if unpredictable) markets, an increase in resource status, and apparent viability (persistence with some replacement) of the fleet. However, the fishery has not changed as anticipated by, or as a direct result of, the Plan. The resource prospect improved during the Plan, but misreporting has precluded an assessment of stock status recently (Stephenson et al. 1991, 1992). Markets improved with the advent of the roe fishery (supported also by OSS and traditional domestic processor demand), but in the 1990's that is proving to be a volatile fishery.

With respect to fleet configuration, forty vessels remain (from a total of 49 in 1983), and a number of the older vessels have been replaced. Potential for further fleet reduction during the Plan was diminished due to the incomplete implementation of the individual transferable quota management framework, in particular, the lack of effective catch monitoring and enforcement of vessel quotas and perception of incomplete incorporation of property rights (i.e., suspected termination of plan and redistribution of quota after 10 yr). Nowak (1992, p. 183) concluded:

> . . . the plan has had a negligible economic effect on individual purse seiners. There is no apparent evidence of matching fishing costs to the size of quota. Overall, the fleet's increased viability should be attributed largely to misreporting and market forces, not to economic mechanisms implicit in the plan.

In summary, the Scotia–Fundy Region Herring 10-Year Plan continued innovative management involving individual transferable quotas. It was successful in allowing a mechanism for fleet reduction, but was hampered by its form of implementation, including lack of effective monitoring, excessive restrictions on transferability and incomplete perception of ownership. As a result, the fishery was progressing through a passive and naive 10 year Plan founded on early 1980s circumstances. Annual plans over the period adhered to the principles of the Plan in part, but deviated from it on a number of occasions to take into account changing circumstances (including in recent years creative ways by SFHAC to circumvent awkward or unrealistic elements of the plan). The flexibility was desirable, but it undermined explicit goals of the plan.

The development of future management plans for the Scotia–Fundy herring fishery must take into account the lessons learned from recent history. Among these lessons one must conclude that future plans require a long-term perspective but must also have a high degree of flexibility consistent with the adopted principles to permit the management system to react in the short term to anticipated changes in the fishery. The approach should shift from management characterized by reaction under crisis to a more forward looking perspective. This should include thorough discussion of scenarios resulting from management action, and some form of evaluation of the potential impacts. A number of problems and complications of the 10-Year Plan should have been foreseen (market changes, resource fluctuation, etc) within the framework of the Plan and without departing from its stated objectives. One way of facilitating this anticipatory view is through integrated modelling of the biological, economic and management principles and alternatives. Such a model could be used by management to explore public policy options that would permit the industry to adapt efficiently to potential changes in markets, stock abundance, and fleet configurations.

In the context of individual quota schemes, it is important to account explicitly for misreporting and discarding, especially in light of the historical experience in this fishery. Steps must be taken to provide effective penalties for noncompliance, compensation to others and/or, alternatively, to provide incentives to participants within the quota system to comply. This includes cooperation on the part of fishermen, as well as institutional measures to ensure that information is properly collected and reported in a timely manner (Pearse and Walters 1992).

The development of future plans must include all participants in the fishery specifically in a consensus building atmosphere. Unlike the previous Plan's emphasis only on purse seiners, future plans should include all harvesters (seiners as well as weirs and gillnet/inshore fishermen) and processors in a single comprehensive plan built on mutually acceptable principles. As Rettig (1986, p. 31) stated:

> this package must be carefully tailored to the specific fishery, with attention to the biology of the fishery as well as to the social, economic and political environment within which fishermen operate. Finally, fishermen must be involved in a major way with the design. . .

Within broad limits, it would appear to be beneficial for all parties to allow greater "ownership" rights to harvesters for catching herring. This includes freer transferability of quotas than under the previous Plan. More complete powers of transfer will undoubtedly lead to more quota transfers, and ultimately more possibilities to reduce excess capacity in the fishery.

As summarized in this paper the 4WX herring fishery has seen a number of novel management initiatives including limited entry, TACs, and vessel quotas, and through these has been an important case study in fisheries management. It is significant, as pointed out by Halliday and Pinhorn (1985) for example, that the first national allocations in the Atlantic herring fishery in 1972 ". . . broke the dam" of resistance to TAC regulations. Indeed, by 1974 ICNAF had achieved international agreement on TAC levels among member countries for all major stocks within its jurisdiction. Similarly, the implementation of an ITQ system in this fishery has been cited often in the rationale for implementing similar schemes elsewhere. However ITQ management is recent, and there are too few summaries of the performance of this approach to be able to evaluate its overall potential (Copes 1986; Sissenwine and Mace 1992). Clearly further analysis of this type — and especially empirical analysis is required to evaluate and understand the effectiveness of ITQs in modern management systems.

In conclusion, the herring fishery of Scotia–Fundy has many times in the past shown itself through collective action to be adaptable to radically changing environments. Moreover, industry participants have often taken on themselves many of the responsibilities required to ensure its continued viability and contribution to the socio-economic structure of the region. It faces a considerable challenge in preparing for a replacement for the 1983 10-Year Management Plan. On the strength of its participants and with progressive and flexible management, the fishery can be expected to grow and prosper in the future.

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The database is accessible for searches and subscriptions. Persons desiring a printout, a diskette, or a subscription information kit should contact Merridy Bradley, Museum Services, Canadian Heritage Information Network, Communications Canada, 365 Laurier Avenue West, Ottawa, ON K1A 0C8, Canada. Telephone: (613) 992-3333; Fax: (613) 952-2318; e-mail (GEMDES): MUS.SER.

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Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) and their North American fisheries

Dans le Bulletin canadien des sciences halieutiques et aquatiques nº 227

Le flétan de l'Atlantique (*Hippoglossus hippoglossus*) et le flétan du Pacifique (*H. stenolepis*) qui vivent au large des côtes de l'Atlantique et du Pacifique de l'Amérique du Nord ont de nombreuses caractéristiques biologiques en commun. La séparation traditionnelle du flétan en espèces distinctes n'est pas uniformément acceptée. La reproduction et les premiers stades de vie sont presque identiques, bien que la dérive sur une grande distance des oeufs et des larves de flétan du Pacifique et la contre-migration des juvéniles n'aient pas été mises en évidence dans le cas du flétan de l'Atlantique. Les flétans adultes migrant depuis les aires de frai d'hiver situées le long de la partie supérieure du talus continental pour se rendre dans les aires d'alimentation d'été situées sur le plateau continental. Les deux espèces de flétan montrent les caractéristiques semblables du point de vue de l'âge et de la croissance. Elles présentent une sélectivité trophique différent du fait que,comparativement au flétan du Pacifique, le flétan de l'Atlantique de petite taille consomme une proportion plus élevée d'invertébrés et le flétan de l'Atlantique de grande taille consomme une proportion plus élevée de poissons. La gestion du flétan dans l'Atlantique et dans le Pacifique est totalement différente.

AUTEURS

R.J. Trumble, J.D. Neilson, W.R. Bowering, and D.A. McCaughran

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Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) and their North American fisheries

In the Canadian Bulletin of
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No. 227

Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) off the Atlantic and Pacific coasts of North America share many biological characteristics. The conventional separation of the halibut into separate species is not uniformly accepted. Reproduction and early life history are nearly identical, although the extensive drift of Pacific halibut eggs and larvae and countermigration as juveniles has not been demonstrated for the Atlantic halibut. Adult halibut undergo migration from winter spawning grounds along the upper continental slope to summer feeding grounds on the continental shelf. The two halibuts show the same pattern of age and growth. They exhibit different trophic selectivity, as small Atlantic halibut consume a higher proportion of invertebrates as small fish and large Atlantic halibut consume a higher proportion of fish, compared to Pacific halibut. Halibut management in the Atlantic and Pacific varies dramatically.

84 pages

AUTHORS
R.J. Trumble, J.D. Neilson, W.R. Bowering, and D.A. McCaughran

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Reviews/Synthèses

Stephenson, R.L., D.E. Lane, D.G. Aldous, and R. Nowak. Management of the 4WX Atlantic herring (*Clupea harengus*) fishery: an evaluation of recent events **2742–2757**

Announcements/Avis **2758**

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Volume 50, No. 12, December 1993

Volume 50, n° 12, décembre 1993

Balm, P.H.M., and T.G. Pottinger. Acclimation of rainbow trout (*Oncorhynchus mykiss*) to low environmental pH does not involve an activation of the pituitary-interrenal axis, but evokes adjustments in branchial ultrastructure **2532–2541**

Björnsson, B. Swimming speed and swimming metabolism of Atlantic cod (*Gadus morhua*) in relation to available food: a laboratory study **2542–2551**

Skiftesvik, A.B., and Ø. Bergh. Changes in behaviour of Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) yolk-sac larvae induced by bacterial infections **2552–2557**

Jenkins, G.P., M. Shaw, and B.D. Stewart. Spatial variation in food-limited growth of juvenile greenback flounder, *Rhombosolea tapirina*: evidence from otolith daily increments and otolith scaling **2558–2567**

Rodríguez-Ariza, A., J. Peinado, C. Pueyo, and J. López-Barea. Biochemical indicators of oxidative stress in fish from polluted littoral areas **2568–2573**

Hammar, J., P. Larsson, and M. Klavins. Accumulation of persistent pollutants in normal and dwarfed Arctic char (*Salvelinus alpinus* sp. complex) **2574–2580**

DeBlois, E.M., and W.C. Leggett. Match/mismatch between the abundance of marine fish eggs and invertebrate predators: an analysis of *Calliopius laeviusculus* (Amphipoda: Gammaridae) population growth relative to the seasonal spawning cycle of capelin (*Mallotus villosus*) **2581–2590**

Wang, Y., and R.D. Evans. Influence of calcium concentrations on cadmium uptake by the freshwater mussel *Elliptio complanata* **2591–2596**

Polacheck, T., R. Hilborn, and A.E. Punt. Fitting surplus production models: comparing methods and measuring uncertainty **2597–2607**

Pearcy, W.G., J.P. Fisher, and M.M. Yoklavich. Biology of the Pacific pomfret (*Brama japonica*) in the North Pacific Ocean **2608–2625**

Patalas, K., and A. Salki. Spatial variation of crustacean plankton in lakes of different size **2626–2640**

Jackson, D.A., and H.H. Harvey. Fish and benthic invertebrates: community concordance and community–environment relationships **2641–2651**

Wilson, C.C., and P.D.N. Hebert. Natural hybridization between Arctic char (*Salvelinus alpinus*) and lake trout (*S. namaycush*) in the Canadian Arctic **2652–2658**

Cattaneo, A. Size spectra of benthic communities in Laurentian streams **2659–2666**

Playle, R.C., D.G. Dixon, and K. Burnison. Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands **2667–2677**

Playle, R.C., D.G. Dixon, and K. Burnison. Copper and cadmium binding to fish gills: estimates of metal–gill stability constants and modelling of metal accumulation **2678–2687**

Havens, K.E. Pelagic food web structure in acidic Adirondack Mountain, New York, lakes of varying humic content **2688–2691**

Pennuto, C.M., and F. deNoyelles, Jr. Behavioral responses of *Drunella coloradensis* (Ephemeroptera) nymphs to short-term pH reductions **2692–2697**

Meador, J.P., U. Varanasi, P.A. Robisch, and S-L Chan. Toxic metals in pilot whales (*Globicephala malaena*) from strandings in 1986 and 1990 on Cape Cod, Massachusetts **2698–2706**

Tuchman, N.C. Relative importance of microbes versus macroinvertebrate shredders in the process of leaf decay in lakes of differing pH **2707–2712**

Hurley, J.P., and P.J. Garrison. Composition and sedimentation of aquatic pigments associated with deep plankton in lakes **2713–2722**

Fogarty, M.J. Recruitment distributions revisited **2723–2728**

Perspectives

deYoung, B., and G.A. Rose. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland **2729–2741**

(Continued inside back)

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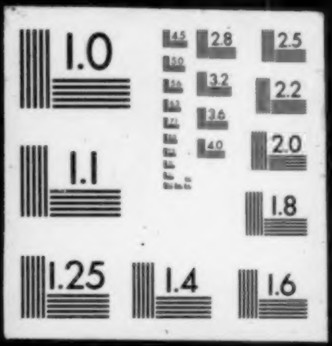
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